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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FORTY-SECOND ANNUAL MEETING OF THE SOCIETY, MEMPHIS, TENNESSEE, DECEMBER 1, 2, AND 3, 1950

Effect of tobacco-mosaic disease on yield of tomato. ALEXANDER, L. J. Experiments have been conducted to determine the loss in yield caused by the tobacco-mosaic disease of tomato. It was previously reported for 1949 that tobacco mosaic reduced the yield in a glasshouse 13.2 per cent, and that the greatest reduction occurred during the early part of the season. In 1950 a similar experiment with glasshouse tomatoes confirmed the 1949 results. However, in 1950, the experiment was enlarged to include a comparison of the effect of the disease on the two varieties, Strain A Globe and Ohio W-R Globe. Early mosaic infection reduced the total yield 7.9 per cent, whereas in 1949 the reduction was 13.2 per cent. There was no reduction in total yield with Ohio W-R Globe. The early production of both varieties was considerably reduced. Reduction in yield is occasioned by poor fruit set. Plants of the variety Strain A Globe infected January 18 set an average of 2.95 fruits per cluster for the first two clusters, whereas plants infected later, approximately May 15, set 4.55 fruits per cluster. Plants of the variety Ohio W-R Globe, infected January 18, set an average of 3.35 fruits per cluster for the first two clusters, whereas plants infected later set an average of 4.6 fruits per cluster.

Methods for determining pathogenicity of Sclerotinia trifoliorum on alfalfa and Rhizoctonia solani on Lotus. ALLISON, J. LEWIS, AND CLARENCE H. HANSON. Two pathogenic fungi of economic importance in North Carolina are *Sclerotinia trifoliorum*, causing crown and stem rot of alfalfa during the winter, and *Rhizoctonia solani*, causing foliage blight on *Lotus* in the summer. Methods were developed for determining the reaction of alfalfa and *Lotus* to the respective organisms under controlled conditions. Except for temperature (60°–70° F. for *S. trifoliorum* and 80°–95° F. for *R. solani*), the procedures were identical. Young plants were inoculated with a composite of pathogenic isolates of the respective fungi. Agar cultures were mixed with water in a blender and the suspension of mycelial and sclerotial fragments was sprayed on the foliage of plants. Inoculated plants were placed on benches under plastic cloth tents and kept moist by means of fog-producing apparatus. Initial symptoms produced by each pathogen appeared in 6–10 days and final disease readings were made 30 days after inoculation. Seventy-five strains of alfalfa, 90 foreign and domestic collections of *L. uliginosus* and *L. corniculatus*, and 11 other species of *Lotus* were tested. The majority of plants in each was highly susceptible to the respective pathogens. The small number of plants which appeared resistant are being subjected to further testing for possible use in the legume improvement program.

Partial purification of carnation-mosaic virus. AMES, RALPH W., A. E. VATTER, JOHN J. SCHOLZ, AND H. H. THORNBERRY. Virus particles of carnation-mosaic virus have been demonstrated by electron microscopy of the sediments from infectious plant juice (King Cardinal variety cuttings) at pH 7.2 in 0.1 M. phosphate buffer ultracentrifuged at 15,000 times gravity. Juice from healthy plants (seedlings) treated in a similar manner did not reveal these particles. From palladium-shadowed (25° angle) electron micrographs at 36,000 × (50 kilovolts) and a projected magnification of 400,000 for measurements, the virus appears to be spherical and approximately 31 mμ in diameter. The sediment at 1×10^{-3} dilution induced the typical disease in carnation and in *Dianthus barbatus* L. after inoculation. Sediment from healthy juice at 1: 10 dilution was not infectious. It is concluded that the infectiousness of carnation-mosaic virus is associated with the demonstrated particles.

The Phytophthora cinnamomi problem in pineapple fields of Hawaii. ANDERSON, E. J. *Phytophthora cinnamomi* Rands is present in much of those pineapple lands of Hawaii where annual rainfall exceeds 50 to 60 inches. The fungus is capable of complete destruction of extensive plantings and seriously reduces yields in much of the infested area. Roots, growing points of stems, and green fruits may be attacked. Destruction of roots accounts for the greatest damage. Infection in stem tips, called heart rot, reduces stands and is always accompanied by root rot. Green fruit rot is comparatively rare. Distribution of rainfall during the year is more important than total annual rainfall in determining severity of the disease. The fungus persists in comparatively dry soil without appreciable infection and attacks severely during periods of prolonged wet weather. Most damage results when soils remain saturated, or nearly so, for several weeks. Gentle slopes and flat areas with very tight subsoils are most seriously affected. The fungus has been

recovered from the surface inch as well as greater soil depths after prolonged wet weather, but only from deeper soil after prolonged dry weather. It has been recovered from a depth of 27 inches in very compact subsoil.

Effect of nutrient salt concentrations on infection of pineapple roots by Phytophthora cinnamomi. ANDERSON, E. J. *Phytophthora cinnamomi* Rands attacks and rapidly rots roots of pineapple grown in infested tap-water culture. This appears to be due to infection by zoospores. Rooted shoots were transferred to water cultures of various nutrient salt concentrations infested by addition of a small amount of naturally infested field soil. The number of infected roots decreased as the total nutrient salt concentration in solution increased up to about 1400 p.p.m. in "balanced" solutions. "Unbalanced" solutions of 1000 p.p.m. total salt concentration of which potassium made up 381 p.p.m. prevented infection for 2 weeks and gave only 7.5 per cent infected roots after 1 month as compared with 100 per cent infection after 11 days in a "balanced" solution of equal total salt concentration but only 146 p.p.m. potassium. Infected roots rotted at about the same rate regardless of solution concentration. Root growth in noninfested cultures was about equal regardless of solution concentration. Fertilization of pot cultures in accordance with solution culture results delayed or prevented severe root rot. Leaching of soils in high rainfall areas precludes control by means of heavy fertilization in the field.

Lespedeza diseases in Louisiana. ATKINS, J. G., JR. A severe dying of lespedeza was observed in nursery and field plantings in 1949 and 1950. Affected plants have yellowish and sparse foliage, wilt, leaf blight, root rot, basal stem rot, and stem girdling. *Sclerotium rolfsii*, *Rhizoctonia solani*, and the alfalfa hopper, *Stictocephala festina*, were each capable of severely damaging or killing Korean plants, *Lespedeza stipulacea*. Isolates of *S. rolfsii* from Ladino clover and lupine were also pathogenic. *S. rolfsii* severely damaged Korean but not Kobe lespedeza, *L. striata*, in nursery plantings. Isolates of *Rhizoctonia* from lespedeza leaves, roots, stems, and seed were pathogenic on Korean plants. Lespedeza seed planted in soil artificially infested with *R. solani* failed to emerge; isolates of *Colletotrichum*, *Sphaeropsis*, and *Sclerotium bataticola* caused only slight stand reductions. Plants girdled by hoppers had more injury when *Colletotrichum*, *Sphaeropsis*, and *S. bataticola* were present. Upon inoculation, *S. rolfsii* caused basal stem rot and rapid wilt of Korean lespedeza, and *R. solani* caused damping-off, root and basal stem rot, leaf blight, and wilt. Plants girdled by hoppers had yellow and sparse foliage, slow wilt, and a root or basal stem rot associated with weakly pathogenic fungi.

Investigations on a mosaic of cantaloupe in South Carolina. AYCOCK, ROBERT. A cantaloupe mosaic caused by an unidentified virus occurs extensively in South Carolina. The virus is readily transmitted to healthy cantaloupe plants by the leaf-rubbing technique. Symptoms include veinclearing, veinbanding, and mottle. Infection of cucumber, squash, and watermelon is also easily obtained. On inoculated cucumber plants veinbanding with little or no mottle occurs; ringspotting, oak-leaf patterns, and mottle develop on squash. Small local, necrotic lesions are produced on the inoculated leaves of watermelon. Attempts to induce infection in noncucurbitaceous hosts which are susceptible to the known strains of cucumber mosaic virus have been unsuccessful. In the field the disease has been noted only on cucumber and cantaloupe.

Fungitoxicity of cyanamides in relation to pH of liquid media and organic content of soils. BASKIN, A. DAVID. Four synthetic soils in which organic matter varied from 0.29 to 5.08 per cent were infested with *Rhizoctonia solani*, *Actinomyces scabies*, *Sclerotinia sclerotiorum*, or *Fusarium bulbigenum* var. *niveum* and were treated with $CaCN_2$ at rates equivalent to 500–6000 lb. per acre. A high organic content enabled the fungi to survive relatively high concentrations of cyanamide, the relationship being characteristic and constant for each fungus. Potato-dextrose broth with 0.7 per cent KCNO or NaHNCN by weight, or more, had a pH of 9.3 and was lethal to *F. bulbigenum* var. *niveum*. The fungus grew if such a solution was acidified, and growth increased with decreasing pH. Acidification altered the toxicity of cyanamides, probably by hastening their decomposition. Manometric studies indicated that buffer composition as well as pH must be evaluated. Addition of acid buffers to KCNO solutions resulted in rapid evolution of CO_2, even in absence of the fungus. Respiration of *Fusarium*, which occurred normally between pH 3.7 and 7.8, was inhibited by acetate buffers, whereas citrate and phosphate buffers of the same pH permitted oxygen uptake.

Translocation of poisons, dyes, and radioiodine, and its relation to oak wilt. BECKMAN, C. H., AND J. E. KUNTZ. The local spread of oak wilt through natural root grafts was clarified by tracing similar tree-to-tree movements of sodium arsenite, ammate, and radioactive iodine^{131}. In one of four jack-oak plots, when a single tree was treated with

arsenite, five trees were killed. When these were treated, 21 additional trees died. When these in turn were treated, another ten trees died. Some trees by-passed initially developed symptoms after later treatments. Such movement followed closely the pattern of local spread in oak wilt. Radioactive iodine^{131} moved within 6 hr. to three trees respectively 2, 5, and 12 ft. from the nearest treated tree. Grafts from bur to bur or white to white oak were found only occasionally. In 31 plots of mixed bur and jack oaks, only one case of movement and one graft were found between these species. Lateral translocation from local treatments was extensive within individual jack oaks, but was limited within individual burs and whites. Upward movement of radioactive iodine^{131} was as rapid as 12 ft. within 15 min. in jack and bur oaks. Downward movement was limited except where root grafts occurred.

Distribution of hyphae of several plant-pathogenic fungi in leaf tissue. BELL, FRANK H. Observations have been made of the mycelia of several species of fungi present in and on host leaves. This study has been made possible by the development of a simple two-step staining method. Leaf chlorophyll was removed and the mycelium stained by leaving the leaf pieces overnight in a chloral hydrate solution (5 parts chloral hydrate to 2 parts water) containing 0.1 per cent aniline blue. Excess stain was removed by clearing several hours in the chloral-hydrate solution. Better clearing and staining was obtained in approximately 4 hr. by twice subjecting the leaves in the staining solution to a partial vacuum, and then placing them in a 60° C. oven. The mycelia remained stained for a few weeks in chloral-hydrate mounts ringed with a mixture of equal parts beeswax and gum dammar. The hyphae of *Helminthosporium avenae* branched relatively seldom, and consequently the mycelia were less dense than those of *Melampsora lini, Puccinia graminis avenae,* and *Uromyces appendiculatus.* The mycelia of flax rust were relatively small, and the hyphae branched profusely among the small host cells. Flax-rust and bean-rust mycelia were nearly circular; those of the cereal rusts were oblong to diamond-shaped.

Size and structure of the various infection-type mycelia produced by the wheat stem-rust fungus. BELL, FRANK H. A study has been made of 6-day-old mycelia of wheat stem rust after they were stained by aniline blue contained in a chloral hydrate solution. The average length of mycelia of a particular infection type varied with the wheat variety, the environmental conditions, and the race of rust used. However, the average lengths of mycelia that produce a susceptible reaction (infection types 3 and 4) were nearly always greater than those of mycelia that result in a resistant reaction (infection types 0, 1, and 2). In some cases this difference in average size between these two classes of mycelia was not great, but many 3- and 4-infection-type mycelia were two or more times as long as the largest 2-infection-type mycelia. Mycelia of the 2-infection type often averaged one and one-half to two times as long as 1-type mycelia. No constant size difference has been found between the mycelia of infection types 3 and 4. The margins of resistant-class mycelia were more uniform in outline and contained more hyphae per unit area than those of susceptible-class mycelia. The average lengths of mycelia that produced a mesothetic reaction were nearly always in the size group for the susceptible class. Measurements at other stages indicate that the relative difference in size between mycelia in the resistant and susceptible classes increased with the age of the mycelia.

An obscure disease of banana. BENSAUDE, MATHILDE, AND ANNA E. JENKINS. In her inventory of the diseases of agricultural plants on St. Michael Island, Azores, the senior writer reported a "verruga," or wart, of banana that according to the literature available at that time (1927) appeared to be new. The disease was said to be present but mild in Madeira. Several years ago we became aware that the malady, in its symptoms and its association with humid conditions, at least simulates a *Sphaceloma* disease. During 1949–1950 this has been corroborated by new field observations on St. Michael and by laboratory study of representative material collected. Showing that the disease affects young growth, as do *Sphaceloma* diseases, were such examples as very small water-soaked spots on fruits 5 to 7 cm. long with the floral parts still attached. On somewhat larger fruits each spot had its tiny corky center. In 1927, when the parasitic nature of the disease could not be demonstrated, excessive moisture was suggested as the cause. Its exact nature is still obscure; however, our recent observations suggest that it may be caused by a parasite which produces abundant inoculum.

Gladiolus viruses. BERKELEY, G. H. It has been demonstrated that Picardy and other varieties having the common mosaic mottle were infected with strains of the tobacco ring-spot virus, and that a single plant of the variety Mrs. Marks Memory, having chlorotic ring and line-pattern symptoms, was infected with a combination of tobacco ring-spot and tobacco-mosaic viruses. Also, an unknown variety was found to be infected with a yellow

strain of the cucumber-mosaic virus. These viruses were transmitted by juice transfer from sprouts 1 to 2 in. long of both planted and unplanted corms, and from tips of leaves up to 12 in. long. Transfers from older plants were less readily obtainable. The identity of these viruses was established by a comparison of symptoms on tobacco var. Harrow Velvet, *Nicotiana glutinosa*, pea var. Laxton's Progress, bean vars. Michelite and Bountiful, snapdragon, tomato var. Bonny Best, cucumber var. Windermoor Wonder, pepper var. California Wonder, and *Datura stramonium*, and by cross-protection tests in the case of the strains of the tobacco ring-spot virus. The cucumber-mosaic and tobacco-mosaic strains produced symptoms similar to those associated with these viruses, except that *Datura stramonium* was killed by the gladiolus strain of the tobacco-mosaic virus.

Control of Rhizoctonia damping-off of Pinus caribaea seedlings. BIRCHFIELD, W., AND G. F. WEBER. *Rhizoctonia solani* Kühn has been the principal causal agent of damping-off of slash pine seedlings in Florida. Infection takes place at germination, and the seedlings are most susceptible during the first 3 weeks of growth. Eleven chemical treatments and four soil treatments were used in control experiments on virgin soil, on soil where nursery stock is being produced annually, and in inoculated soil in the greenhouse. The most effective treatments on virgin soil, in descending order, were Arasan seed treatment on nontreated soil, Semesan on nontreated soil, and Spergon on Tersan-treated soil. The results on the nursery site were, in descending order, no seed treatment on soil treated with sulfuric acid, no seed treatment on nontreated soil, and Semesan on soil treated with formaldehyde. In the greenhouse the best results were obtained with Semesan-treated seed on Tersan-treated soil, followed closely by Spergon-treated seed on Tersan-treated soil.

Linkage groups in Venturia inaequalis. BOONE, D. M. In an attempt to establish linkage groups in the apple-scab pathogen, *Venturia inaequalis*, a genetic study was made using 20 mutant characters, two loci for pathogenicity, and the locus for mating type. Ascospores were isolated, for the most part in serial order, from some 3500 asci and cultured separately to determine segregation of alleles and recombination of loci. Some 2000 other asci were examined microscopically to determine segregation of those mutant factors that induced ascospore abortion. Where possible, genes were located with reference to their centromeres by using Lindegren's method in which one-half the percentage of second-division segregation of a gene pair is taken as the uncorrected distance in cross-over units of the pair from its centromere. Although some studies of recombination of loci have not been completed, there is evidence that marker characters for four chromosomes have been found. Present data indicate five linkage points, including the centromere, in linkage group I, four in group II, three in group III, and two in group IV. The genes conditioning pathogenicity, mating type, and seven of the mutant characters have not yet been located with reference to their centromeres or to loci of other genes.

Collar rot of peanuts, primarily a heat canker. BOYLE, LYTTON W. Collar rot of peanut plants is characterized by lesions of rotting tissue at or above the first node. When collar rot is found in a field, the primary lesions are at a consistent height on the stems and are oriented in one direction, usually west. Fungi were isolated from the lesions, but inoculations with these were negative. Characteristic specimens of collar rot were produced by exposing young plants to radiation from a heat lamp. Data obtained from controlled tests with the heat lamp and from peanut fields during the early season correlate to show that solar radiation is sufficient to burn unprotected stems at the soil level. Seedlings are particularly susceptible to injury before much protection is afforded by their leaves. Laying soil to the row may fold larger plants in such a way that stems do not get full protection by their leaves. All degrees of injury may be found, from readily apparent severe lesions to those not apparent except under magnification. The point of injury may heal and be mistaken for mechanical or insect damage, or it may become infected by weak parasites and collar rot is the result.

Verticillium wilt now recognized as a destructive disease of strawberries in New York State. BRAUN, ALVIN J. Discoloration, stunting, wilting, and dying of strawberry plants has periodically occurred in serious proportions for a number of years in New York State. Culturing from crowns, petioles, and flower stalks of plants having a certain characteristic combination of these symptoms has consistently yielded a species of *Verticillium*. Although Verticillium wilt was first reported as a major disease of strawberries in California in 1932, it has not been recognized as affecting production in the northeastern States. In general, the symptoms of the disease as it occurs in the northeastern States are similar to those reported from California. However, under New York State conditions affected plants invariably have moderate to severe root and crown decay and slight to moderate discoloration of the vascular system, especially at the base of the crown. There is a marked range of variation in susceptibility of varieties commercially grown in the northeastern States.

Control of black rot of grapes without direct coverage of the fruit. BRAUN, ALVIN J., AND FRANK EMERSON. The present recommendations for the control of black rot (*Guignardia bidwellii*) of grapes emphasize the need of thorough coverage of the berries with the fungicide in order to prevent rotting. Experiments and observations demonstrate that where ferbam is used, thorough coverage is not essential. Although the addition of various spreader-stickers increased the deposit and retention of ferbam on the developing berries, their omission did not decrease the control of black rot in Concord vineyards sprayed with ferbam 2–100 in 1949 and 1950. In these tests bunches were bagged at the time of the last application (7 days after bloom) and the bags removed 4 hr. after spraying. Other plots received only the pre-bloom application and one immediately after bloom. The control of black rot on the bunches not receiving a direct application of the fungicide was equal to that obtained on the bunches from vines receiving the 7-day application in the conventional manner. Residue data indicated that redistribution of the ferbam, as such, was not responsible for the control of the disease on the bunches not receiving a direct application of the fungicide.

Gladiolus as a virus reservoir. BRIDGMON, G. H. In a study of the prevalence of bean virus 2 in gladiolus plantings in the vicinity of Madison, Wisconsin, the typical and pod-distorting strains were found commonly in plants showing leaf mottle and flower break. From the same source two other viruses were recovered repeatedly. One of these, by property and cross-protection tests, was a strain of the tobacco ring-spot virus. The other was shown by similar methods to be a strain of cucumber virus 1. The gladiolus strain and authentic green and yellow strains of tobacco ring-spot virus incited symptoms on and were recovered from corn (maize); this and gladiolus are newly recorded hosts for this virus. The cucumber virus recovered from gladiolus did not infect legumes, but it and a typical strain of cucumber virus 1 incited symptoms on corn. Gladiolus is a newly recorded host for cucumber virus 1. A causal relation between any of the viruses and flower break in gladiolus was not established.

Relation of southern bean mosaic to black root. BRIDGMON, G. H. Varieties of bean susceptible to bean virus 4, when inoculated with the virus by the rubbing method, develop systemic mottle. Those rated as resistant develop local lesions on inoculated leaves. When approach grafts between resistant and susceptible plants were made and the latter were inoculated, systemic necrosis of stems, leaves, and pods, not unlike symptoms of black root, resulted on the resistant member of the pair. The symptoms resembled those which have been described when varieties of the Corbett Refugee type resistant to bean virus 1 were inoculated with the latter virus by the approach-graft method. The necrosis incited by bean virus 4 was reddish-brown to black; that incited by bean virus 1, gray to black. Necrosis incited by virus 4 was usually milder than that incited by virus 1. In view of these facts the original description of black root may have referred in part to systemic necrosis induced by the southern bean-mosaic virus.

Fungus parasitism by Rhizoctonia. BUTLER, E. E., AND T. H. KING. Isolates of *Rhizoctonia* spp. pathogenic to roots of strawberries also may parasitize mycelium of certain Phycomycetes: *Rhizopus nigricans* (+ and – strains), *Syncephalastrum* sp., *Mucor mucedo, Cunninghamella* sp., *Helicostylum* sp., and *Phytophthora cinnamomi.* They were unable to parasitize *Thamnidium* sp., *Circinella* sp., *Mucor genevensis, Phytophthora cactorum, P. palmivora,* and *P. fragariae.* Two methods of attack were observed: 1) *Rhizoctonia* penetrated the host mycelium by a few infection hyphae from which limited or extensive internal growth developed, or 2) *Rhizoctonia* hyphae coiled around the host mycelium and penetrated at frequent intervals, with many infection hyphae establishing an extensive internal parasitic mycelium. Invading hyphae replaced the protoplasm of the host mycelium but complete destruction of the host was rare. Initial infection of hosts on potato-dextrose agar occurred in 3 to 5 days at 25° C. *Rhizoctonia* produced more abundant barrel-shaped cells and sclerotia when parasitizing fungi than when growing alone on agar media.

The occurrence of Phytophthora cinnamomi in the soil under pine stands in the Southeast. CAMPBELL, W. A. By the use of the apple method for isolating *Phytophthora cinnamomi* Rands from soil, the relative abundance of the fungus was determined in 61 plots in pine stands outside the littleleaf area in Alabama, Florida, Georgia, Mississippi, North Carolina, South Carolina, and Texas. Twenty-four soil samples were taken from an area ¼ acre in extent in each plot. The fungus was isolated from the soil of 48 per cent of 61 plots, regardless of type of stand, and from 52 per cent of the 50 plots in stands containing shortleaf or loblolly pine, either pure or in mixtures. The fungus was present in the soil in only 14 per cent of the seven plots in stands having slash and longleaf pine. Only 26 per cent of the 30 plots from which *P. cinnamomi* was isolated gave recoveries

from six or more of the 24 samples taken from each plot. In general, although the fungus was isolated from the soil under pine stands outside the littleleaf area, it was present in relatively fewer plots and in relatively smaller amounts than in the soil of plots in the littleleaf area of the South Carolina Piedmont.

Dutch elm disease chemotherapy with Carolate and related formulations. CAROSELLI, N. E., AND A. W. FELDMAN. Carolate (low-magnesium lime, salicylate, and urea), alone and combined with an azo dye, has been compared with related formulations for reducing injury from Dutch elm disease during the past 3 years on more than 2,000 woodlot and estate elms. When woodlot elms were inoculated with *Ceratostomella ulmi* after Carolate application by hydraulic soil impregnation, the disease was arrested in approximately 70 per cent of the trees for more than 2 years, irrespective of application time. The host responds to Carolate by formation of a broad annual ring circumscribing the discolored tissue. *C. ulmi* can be cultured from trees treated 3 years previously with Carolate, indicating Carolate *per se* is not fungicidal in the host, though the azo dye and salicylate are fungicidal at 1: 1,000 and inhibit toxin formation at 1: 50,000. Severe trunk wounding of trees treated and inoculated 2 or 3 years previously caused fresh outbreak of the disease. Resumption of pathogenesis varied with therapeutant formulation, i.e., 70 per cent of the trees succumbed in the low magnesium lime-urea treatment; 40 per cent with the same combination plus azo dye; and 18 per cent with Carolate. Records on 1,100 estate trees treated with Carolate show 40 per cent mortality when less than 5 per cent wilt, and 79 per cent mortality when more than 5 per cent wilt, was present at time of treatment.

The elimination of fireblight-susceptible individuals in interspecific crosses of pear. CARPENTER, T. R. F_1 seedlings of pear crosses involving the parental species *Pyrus ussuriensis* Maxim. and *P. serotina* Rehd. were inoculated with *Erwinia amylovora* (Burrill) Winslow et al., in succulent stem tips with a hypodermic needle, in early growth stages in the greenhouse and in later growth stages in first-, second-, and third-year nurseries, to ascertain the relative merits of inoculations at different growth periods. A higher percentage of susceptible individuals was eliminated in the group originally inoculated in the greenhouse than from similar progenies initially inoculated in the first-year nursery. Seedlings inoculated in the greenhouse demonstrated a greater tendency to gain in resistance and less tendency to shift to different infection classes during the second- and third-year inoculations than the seedlings initially inoculated in the first-year nursery. Seedlings grown in sand at different N and mineral salt concentrations failed to demonstrate great differences in resistance or susceptibility at the different nutrition levels; however, more susceptible individuals were eliminated in standard Hoagland's solution than in solutions of higher or lower concentration. The most favorable temperature found for eliminating susceptible individuals was 25° to 26° C.

Resistance to bacterial blight in soybeans. CHAMBERLAIN, D. W. In 1947, approximately 1200 varieties and introductions of soybeans were tested for reaction to bacterial blight (*Pseudomonas glycinea*). Three highly resistant introductions were selected for study in the greenhouse. They were tested by water-soaking the leaves with the bacterial suspension by means of an atomizer connected to a compressed-air line. The susceptible varieties, Illini and Bansei, were used as controls. The resistant introductions developed only necrotic spots, whereas the susceptible varieties developed spreading, translucent, water-soaked areas within 5 to 7 days after inoculation. Highly diluted inoculum induced slight chlorosis on the resistant introductions; the susceptible varieties, under the same conditions, developed typical blight symptoms. Leaf samples, taken at daily intervals from inoculated leaves of susceptible and resistant plants, were crushed in sterile water and plated on potato-dextrose agar to determine comparative bacterial populations. There was little difference in the rate of multiplication of *Ps. glycinea* in the leaves of resistant and susceptible soybeans until 4 days after inoculation. Subsequently, bacterial populations increased rapidly in the susceptible leaves, in contrast with a slow, limited increase in the resistant leaves.

Relation of specific chemotherapeutants to the infection court. CHAPMAN, RICHARD A. Applied prior to inoculation, chemotherapeutants directly inhibiting pathogen activity must locate near the infection court. Materials applied to a plant's substrate tend to concentrate in the top if mobile, or in roots if substantive. Tests for zones of inhibition about filter paper discs indicate that 8-hydroxyquinoline benzoate is not adsorbed by cellulosic substances, and moves through plants to the top. In practice it is more effective against the elm-twig-invading *Ceratostomella ulmi* than against the tomato-root-invading *Fusarium lycopersici*. In contrast, n-octadecyltrimethylammonium pentachlorophenate (HD3) is tied up by filter paper and must be similarly held by cellulosic substances in plant roots. It is effective against tomato Fusarium wilt but not against Dutch elm dis-

ease. Since n-octadecyltrimethylammonium bromide and n-octadecyldimethylbenzylammonium chloride are ineffective against Fusarium wilt, the activity of HD3 is probably due to the pentachlorophenoxy anion. Commercial sodium pentachlorophenate is not adsorbed in zone of inhibition tests. Thus the n-octadecyltrimethylammonium cation is responsible for the adsorption of HD3. N-(4-nitrophenyl)-3,4-dichlorobenzenesulfonamide is effective against Fusarium wilt and not against Dutch elm disease. It behaves in zone of inhibition tests identically with HD3.

Physiologic specialization in cereal smuts. CHEREWICK, W. J. During the past 4 years, more than 1,000 collections of cereal smuts have been subjected to identification tests on a series of differential hosts at the Dominion Laboratory of Plant Pathology at Winnipeg. As a result of these tests and others made at this laboratory in preceding years, the following numbers of races have been identified: *Ustilago hordei*, 13; *U. nigra*, 11; *U. nuda* and *U. tritici*, 10 each; *U. kolleri*, 4; and *U. avenae*, 17. The addition of two oat varieties, Mabel and Beacon, to the 10 selected by Holton and Rodenhiser increases the number of races of the two latter smuts to 10 and 23, respectively. The addition of two barley varieties, White Hulless and Newal, to the group selected by Tapke increases the number of races of *U. hordei* and *U. nigra* to 16 and 14, respectively. In the bunt fungi, three races of *Tilletia caries* and two of *T. foetida* have been identified.

Occurrence of black rot of oranges in Florida. CHRISTIE, J. R., AND J. H. OWEN. *Alternaria citri* Ellis & Pierce ordinarily causes highly colored fruit with blackened stylar ends and core rot. This internal decay is sometimes outwardly masked. Masked decay sometimes contaminates frozen orange-juice concentrate, and suspected fruit lots are rejected. The fungus invades oranges through wounds, causing black rot of the mature fruit, both on the tree and in the moist chamber. Pineapple and Jaffa orange flowers, inoculated on the tree with spore suspensions under various conditions, did not become infected. The disease was produced only on injured, tree-attached oranges ¼–3 in. in diameter with spore suspensions and mycelium-covered agar disks placed in cuts into albedo and pin pricks into epidermis. Detached mature fruit in the moist chamber was infected only through wounds. On Czapek's agar cardinal temperatures are 5°–28°–36° C., and pH range includes 3.0 through 9.5, optimum growth at pH 7.0.

Breeding behavior and growth responses resulting from the transfer of wildfire resistance from Nicotiana longiflora to N. tabacum. CLAYTON, E. E., H. E. HEGGESTAD, J. G. GROSSO, D. R. BOWMAN, AND E. O. SCHNEIDER. After making the initial cross, one backcross to tobacco, plus selfing, produced a stable resistant line (T.L. 106) with a 24_{II} chromosome condition, the same as cultivated tobacco. This line was not commercially desirable, but occasional lines of apparent commercial value were obtained as early as the second backcross. Regular segregation was not secured until after the fifth and sixth backcrosses. Then, out of a group of eleven F_2 populations, seven gave excellent fit for a simple ratio of 3 resistant to 1 susceptible, indicating that longiflora wildfire resistance is controlled by a single pair of dominant genes. Extensive field studies have shown that wildfire-resistant lines compared with susceptible check varieties in absence of disease damage tend to yield slightly more and to grade out slightly better. There seems to be a linkage between resistance and reduced nicotine content. Thus, nicotine determinations for a group of resistant lines ranged from 1.83 to 2.88 per cent. The corresponding figures for the checks of commercial tobacco were 3.60 to 4.23 per cent. In general, the addition of wildfire resistance appears to favor improved agronomic and quality characters.

Wilt of red clover seedlings. CRALL, J. M. Wilting, with vascular and cortical discoloration of the taproot, occurred commonly in first-year field plantings of red clover in Iowa in 1949. Vascular discoloration of 5–20 per cent of the plants was observed frequently after late June. *Fusarium oxysporum* was isolated consistently and *F. solani*, *F. moniliforme*, and *F. roseum* frequently from the diseased tissues. Pathogenicity tests were made in artificially infested soil in the greenhouse at 60°–70° F. Ten of 22 isolates of *F. oxysporum* reduced stands of Emerson red clover 35–62 per cent after 3 months. Plants wilted and developed vascular discoloration typical of the Fusarium wilts. Several isolates of *F. solani* and *F. moniliforme* caused slight stand reductions. In a second experiment carried out in the greenhouse in the summer of 1950 one isolate of *F. oxysporum* caused stand reductions after 3 months as follows: Emerson, 33 per cent; Midland, 30 per cent; Kenland, 18 per cent; "common," 36 per cent. Kenland was most resistant and "common" most susceptible to all pathogenic isolates. There was no evidence of physiological specialization among six isolates of *F. oxysporum*.

The control of soybean chlorosis in rice-growing areas of Arkansas by potassium applications. CRALLEY, E. M., AND R. L. BEECHER. Soybean chlorosis, resembling K-deficiency

in symptoms, has been observed for several years in some of the rice-growing areas of Arkansas. The disease was first observed in fields where soybeans followed rice in the rotation system and was most conspicuous in narrow bands in the fields, which coincided with the location of the leveled rice levees. The disease has gradually increased in importance to the point where large acreages are involved, and 75 per cent of plants in certain fields show deficiency symptoms. Soil analysis showed that the soils in the areas involved were alkaline (pH 7.0 to 8.2) and were low in replaceable K. The soils also showed exceptionally high concentrations of Ca and Mg (replaceable Ca to K ratios of more than 60 to 1). Fertilizer tests were conducted using K alone and in combination with N, P, and minor elements. The results showed that K, alone (30 to 80 lb. per acre of available K_2O) or in combination with N and P, prevented chlorosis when applied at the time of planting and resulted in marked recovery of the plants when applied as a side dressing after the plants were showing deficiency symptoms.

Control of Helminthosporium oryzae seedling blight of rice by seed treatment with Ceresan M. CRALLEY, E. M., AND R. G. FRENCH. Zenith and Bluebonnet rice seed, severely infected with *Helminthosporium oryzae*, were treated with Ceresan M at rates of ¼, ½, ¾, 1, and 1½ oz. per bu. and planted in greenhouse flats 1 day, 2 weeks, and 4 weeks after treatment. Significant reduction in seedling blight was obtained with all rates of treatment, on both varieties and at all dates of planting. However, the lowest percentage of blight was observed when the seed was planted 4 weeks after treatment. A gradual increase in mercurial toxicity was observed on the seedlings as the treatment rate was increased from ¾ to 1½ oz. per bu. A comparison was made of Ceresan M (½ oz. per bu.) and Arasan (1½ oz. per bu.) on the control of *H. oryzae* seedling blight on Bluebonnet rice. Ceresan M gave definitely better control than Arasan. The number of healthy seedlings observed 2 weeks after planting from lots of 100 seeds were: 5 for no treatment, 16 for Arasan, and 62 for Ceresan M. These results on the control of *H. oryzae* seedling blight, coupled with promising field tests and low cost of treatment, rate Ceresan M as a strong contender in the field of rice seed-treatment compounds.

Chemotherapeutant effect of certain aryloxyaliphatic acids. CROWDY, S. H. In tests of fungitoxicity of materials known to be capable of translocation in plants, preliminary trials on agar media against *Nectria galligena* mycelium showed that naphthoxy-acids were more fungistatic than phenoxy-acids and that increase in the chlorination of the nucleus added to this effect. Subsequently, greenhouse trials were made with the more highly chlorinated phenoxy-acids, excluding those showing strong growth-regulating activity on higher plants. The test materials were added to the soil in which bean plants were growing, injected into the stem, or sprayed onto the leaves, which were later inoculated with spores of *Botrytis fabae* or *B. cinerea*. In all plants leaf infections developed, but the therapeutant effect of the acid showed in the diminished rate of growth of the lesions on the treated plants. The effect was assayed either by estimating percentage leaf area affected or by measuring individual lesions. Using these criteria, 2-4-6-trichlorophenoxyacetic acid, pentachlorophenoxyacetic acid, and pentachlorophenoxy*iso*butyric acid produced average reductions in disease of 30–40 per cent.

Some metabolic and morphologic changes in germinating conidia of Fusarium. COX, C. E., AND H. D. SISLER. Conidia of *Fusarium roseum* washed free of the medium on which they were produced and suspended in modified Czapek's solution at pH 6 and at 30° C. have a low rate of endogenous respiration which is maintained for a long period of time, but which does not support germination. During a period of about 1 hr. after an exogenous source of carbohydrate is supplied, a sharp increase in the rate of O_2 uptake occurs and is accompanied by swelling and other morphological changes. This is followed by a period of 60–90 min. during which the rate of O_2 consumption remains essentially constant. Then the rate of O_2 uptake again begins to increase and germ tubes appear. The rate of respiration progressively increases from this point onward as additional conidia germinate, as germ tubes elongate, and mycelial growth begins. Conidia deprived of exogenous carbohydrate undergo essentially no swelling and there are few, if any, spores that germinate. Ethyl alcohol and certain other compounds may replace sugar as an exogenous source of C. In addition to C, certain inorganic salts are also required for normal germination of *F. roseum*. Respiration of *F. oxysporum* f. *batatas* closely parallels that of *F. roseum*.

Artificial inoculation of peach seedlings with Xanthomonas pruni. DAINES, ROBERT H., AND L. F. HOUGH. Nearly 2,000 peach seedlings from 19 controlled crosses involving 21 different parents were artificially inoculated during February of 1950. The seedlings were germinated in fresh Vermiculite, and given a full nutrient solution and supplemental illumination to force vigorous growth until the 6- to 12-leaf stage. When the stomata

were open (from about 11 a.m. to 3 p.m. on bright days), the seedlings were uprooted and the roots rinsed free of Vermiculite in water at 23–25° C. The seedlings were tied in convenient bundles and the tops immersed in a heavy suspension of *Xanthomonas pruni* in a large vacuum desiccator. They were subjected to 5 in. of vacuum which was maintained for 2–3 min. while the suspension was agitated to wash the air bubbles from the stomata. The vacuum was released abruptly and the procedure repeated. Conspicuous water-soaked areas were produced on nearly all leaves. These usually disappeared within 2–6 hr. The seedlings were heeled in flats of moist Vermiculite and placed in a moist chamber kept at more than 90 per cent relative humidity until they were planted in soil in the greenhouse. Severe infection was apparent on nearly all seedlings 4–6 weeks after inoculation. Segregation for moderate levels of infection was observed in a few progenies.

Stem anthracnose of bean in Florida. DARBY, JOHN F. Pure cultures of *Colletotrichum truncatum* (Schw.) A. & M. were maintained on fresh, uncooked Fordhook Lima beans aseptically removed from the pods. Spores germinate in 1–2 hr. at 10°–33° C., with 27°–28° C. as the apparent optimum. Germinating spores on the leaves of Henderson's bush Lima bean, *Phaseolus limensis* Macf., usually become septate and send out germ tubes varying in length up to 150 μ. Dark brown, clavate to globular, thick-walled appressoria form at room temperatures at the end of the germ tubes 20–24 hr. after germination. A prominent septum usually separates the germ tube from the appressorium. Infection hyphae develop from the under surfaces of the appressoria, penetrate the cuticle, and reach the sub-epidermal cells in 100 hr. Most of the early growth occurs in the epidermal area. The stromata develop in this area and acervuli 200–300 μ in diameter are formed in and above the epidermal layer in 5–7 days. Growth for 12 days is listed in descending order on Difco agars: potato-dextrose, cornmeal, prune, bean pod, Lima bean, tomato, Russell double sugar, nutrient, and yeast-dextrose agars. The cardinal temperatures on Difco 2 per cent potato-dextrose agar were 5°–27°–37° C.

Microflora associated with surface-sterilized wheat seed. DAVIDSON, R. S., AND DEN SHEN TU. Microflora associated with surface-sterilized seed of four varieties of soft red winter wheat, Trumbull, Thorne, Butler, and Seneca, from nine locations in Ohio, was studied for 3 years. The predominating organisms were *Fusarium graminearum* Schw., *Alternaria* sp., and a white unidentified bacterium. No significant difference was found among the four varieties for the percentage of seeds bearing *Fusarium*. The percentage of Butler with *Alternaria* was significantly higher than for the other three varieties. The percentage of Thorne bearing *Alternaria* was significantly greater than for Trumbull. The low percentage of seeds of Butler with bacteria associated was highly significant. The correlation between the three organisms was positive and very close to significance for *Fusarium* and *Alternaria* in relation to the varieties. A nonsignificant negative correlation occurred between *Fusarium* and bacteria, and *Alternaria* and bacteria for the varieties. A significant negative correlation between *Alternaria* and bacteria occured for stations and years. The seeds bearing *Fusarium* were not independent of those with *Alternaria*, but were independent of those with bacteria. Seeds of Thorne and Seneca bearing *Alternaria* were not independent of those with bacteria; however, seeds of Trumbull and Butler bearing *Alternaria* were independent of those with bacteria.

Phomopsis-blight-resistant eggplants. DECKER, PHARES. In 1940 several varieties of eggplant from India were reported to be highly resistant to Phomopsis blight. These varieties were of no commercial value because of undesirable characteristics—a low, spreading growth habit, large spines on the stems, leaves, and calyxes of the fruit, and variable fruit color, predominantly green to yellow. These resistant varieties were crossed with the commercial varieties. Resistant selections were made from subsequent generations and backcrossed to the commercial varieties to fix desirable plant and fruit characters in a true-breeding, disease-resistant commercial variety adapted to Florida conditions. Two Phomopsis-resistant varieties have been developed and released. One of these, Florida Market, is a selection from Fort Myers Market by an Indian variety, five times backcrossed and five times selfed. It is similar to the Fort Myers Market type. The other one, Florida Beauty, is a selection from Black Beauty by an Indian variety, six times backcrossed and six times selfed. The plant type is similar to Fort Myers Market with fruit characters more as in Black Beauty. Both varieties have performed satisfactorily in all vegetable-growing areas in Florida.

Trichothecium fruit rot of glasshouse tomatoes. DEEMS, ROBERT E. A minor fruit-rot disease of *Lycopersicon esculentum* Mill. has been collected from plants in various Ohio glasshouses. The disease first appears as a firm, dark-brown rot, although in nature secondary organisms generally become quickly established, resulting in a soft, watery, dark-brown to black rot. As the rotted condition ages, a surface sporulation of powdery

pink-orange conidia is evident. The causal organism was *Trichothecium roseum* Link. Primary invasion occurs only following fruit injury. Available evidence indicates that the disease may be economically important only when tomatoes are cultured under moist conditions and at temperatures near 19°–22° C. Under these conditions, *T. roseum* can also cause a blossom-blight disease. Cross-inoculation investigations resulted in rots of apples, bananas, peaches, pears, and quinces.

Strawberry viruses in eastern United States, as expressed when transmitted to Fragaria vesca. DEMAREE, J. B., AND C. P. MARCUS. More than 800 strawberry plants were grafted to *Fragaria vesca* in 1950, including 53 varieties and selections taken from 20 States. A large percentage of the plants thus indexed gave virus reaction in *F. vesca*, two or possibly three types of symptoms appearing in the indicator plants. These types are tentatively listed as virus 1, virus 2, and virus 3 but have no relation to similar designations used by other investigators. Virus 1: Petioles erect; leaves small, distorted, asymmetrical, puckered, spotted, and streaked with yellow; plants moderately dwarfed. Considerable variation of symptom characters. Distribution limited. Virus 2: Petioles of infected young runners of *F. vesca* plants short and spreading; leaves symmetrical, smooth, light yellow, sometimes faintly mottled; young leaves of old plants when infected had strong epinastic tendencies and old leaves became horizontal. Symptoms in both young and old plants later changed—petioles upright, thin, and short; leaves small without distortion or spotting; gradually many bud proliferations developed from the stem, forming multi-crown plants. Distribution widespread over eastern United States and symptom variations in *F. vesca* were slight. Virus 3: When yellows-infected plants from Oregon were indexed to *F. vesca*, symptoms developed similar to our virus 1 type, except that dwarfing was more accentuated, and plants were more yellowed and spotted. A combination of virus expressions of types 1 and 2 was frequent.

Response of pea varieties to dry and slurry methods of seed treatment. DEZEEUW, D. J., AND AXEL L. ANDERSEN. Response of pea varieties to Ceresan M (7.7 per cent ethyl mercury p-toluene sulfonanilide) varied with the method of applying the fungicide to the seed. Dry applications of 4 oz. per 100 lb. of seed resulted in significant stand increases, whereas stands of many varieties were reduced significantly with the same rate of application of the fungicide in a water slurry. In separate tests, stands of Little Marvel, Wisconsin Perfection, and Large White Marrowfat were significantly increased when the fungicide was applied at the rate of 2 oz. per 100 lb. as a dust, in a water slurry, or in a 5 per cent methyl cellulose slurry. With the same treatments, stands of Alaska were normal after the dust treatment, only slightly reduced by the methyl cellulose slurry, and substantially reduced by the water slurry. Plants from seeds injured by the water slurry were stunted, lacked secondary roots, and had shortened and browned radicles —all symptoms of typical mercurial poisoning. It is probable that the water in the slurry allows the toxic material to reach the embryo during the period between seed treatment and planting, since seed planted immediately after treatment was not injured.

Multiplication of Bacterium tabacum in leaves of Nicotiana longiflora. DIACHUN, STEPHEN, AND JOSEPH TROUTMAN. Most plants of *Nicotiana longiflora* are highly resistant to wildfire. Commercial types of burley tobacco resistant to wildfire are being developed from crosses between *N. longiflora* and *N. tabacum*. Leaves of burley tobacco (susceptible) and of *N. longiflora* (resistant) were inoculated by water-soaking: a stream of inoculum was forced from a hypodermic syringe against the lower leaf surface and entered through the stomata. Twenty-four-hour broth cultures diluted 1: 100, 1: 1000, and 1: 10,000 were tested. A solid stripe about 2 cm. wide was water-soaked on both sides of and adjacent to the midrib. At intervals of approximately 24 hr., discs 5.5 mm. in diameter were cut out with a cork borer and crushed in sterile water. Dilution plates were poured to estimate the number of colonies of bacteria present inside the discs of leaf tissue. In burley tobacco the number of bacteria in the tissues increased rapidly the first few days, then dropped off as the tissue collapsed, died, and dried. In *N. longiflora* the number of bacteria increased the first few days but did not reach the high levels present in burley. After a few days the number of bacteria dropped off. No symptoms developed in *N. longiflora*.

Continuous gamma radiation suppresses crown-gall formation in tomatoes. DIMOND, A. E. Bonny Best tomatoes inoculated with *Agrobacterium tumefaciens* and then receiving a dosage of 30,000 r of gamma radiation from Co^{60} at a continuous dosage rate of 80r/hr. did not develop galls, whereas check plants did. Different lots of plants were inoculated well before irradiation, just before, and just after irradiation was completed. Galls were suppressed in all cases. Bacteria capable of inducing galls could occasionally be isolated from such irradiated plants. The extent of gall suppression decreased with dosage rate

and galls formed almost normally at 5 r/hr. Bacterial cultures subjected to 50,000 r of gamma radiation at 80 r/hr. were still capable of producing normal galls in nonirradiated plants. Plants surviving 30,000 r of gamma radiation had decreased terminal growth, lack of central parenchyma in new growth, poorly developed leaf laminae, and very poor root development. Thus extent of gall suppression is a measure of radiation damage to the host, and probably results in part from a decrease in ability of the plant to undergo chromosomal and cell division and cell enlargement.

The chemotherapeutic properties of two compounds against Fusarium wilts. DIMOND, A. E., AND R. A. CHAPMAN. 4-Chloro-3,5-dimethylphenoxyethanol (code No. 1182) and 2-norcamphane methanol (code No. 1207) have pronounced ability to eliminate incipient infections by *Fusarium dianthi* on carnations and *F. lycopersici* on tomatoes. In controlled greenhouse experiments, these compounds were applied in aqueous solution to the substrate of tomato plants and were absorbed through the roots. After inoculation with *F. lycopersici* such plants have repeatedly been low in disease grade, whereas check plants were heavily diseased. Under these conditions, disease grade was linearly related to logarithm of the dosage applied for each compound. To be effective, treatment must be made serially, in advance of infection. The therapeutic effect is short-lived. With *F. lycopersici* these materials are more inhibitory to mycelial growth than to spore germination. Each acts inside the host to prevent establishment of infection through mycelial growth. Number 1182 has a formative effect upon tomatoes; No. 1207 has a characteristic odor which it imparts to foliage of plants absorbing it. These can be used to trace distribution and period of activity in the plant. Number 1182 has given more consistent protection than No. 1207.

Helminthosporium crown and culm rot of Southland Oats. EARHART, ROBERT W. In 1949–1950 severe lodging occurred in a new oat variety, Southland (CI 5207), released by the Florida Agricultural Experiment Stations. In one field, 50 per cent of the plants were so lodged and damage to the crown and lower sections of the culms resembled that caused by *Helminthosporium victoriae,* although this variety had been resistant in previous tests. A small, dark-spored Helminthosporium with thick, tapered walls was isolated in every case from these infected plants. Morphologically, the fungus isolated had certain characteristics common to *H. sativum* and others to *H. victoriae;* however, pathologically it does not fit either species. Experimental data indicate that severe infection can be induced by foliage inoculations, with complete killing of the foliage in 4 to 7 days. Foliage symptoms are similar to those described for *H. victoriae;* generally there is severe necrosis and a withered or scorched appearance of infected foliage. Present data indicate that although isolates from different locations vary to a small degree in their pathogenicity on Southland, sister conidia within any isolate do not. It was also determined that there are differences in host varietal response to single-spore isolates of the fungus.

Occurrence of abnormal cotton bolls in Arkansas. EMGE, ROBERT G. During the early part of July abnormal bolls were seen in three counties in southeastern Arkansas, and later in experiment station plots at Fayetteville. This abnormal growth has been given the name "intercapsulary carpels" by workers at the Stoneville (Miss.) station. The condition is caused by the development of supernumerary carpels within the formed boll. As these carpels develop, the boll is split, allowing saprophytic organisms to enter and cause boll rots. The abnormal bolls were found in varying amounts in several varieties. While this condition may be genetic in character, it is believed that climatic factors affect its development. Rainfall in Arkansas this season was much above the average and temperatures were below normal.

Strains of the cucumber-mosaic virus. FAAN, HWEI-CHUNG, AND JAMES JOHNSON. Isolates of the cucumber-mosaic virus from widely separated localities in Wisconsin and from 31 plant genera were compared in the greenhouse on tobacco and other plants. Some isolates from other States were also included in the trials. From 360 isolates, eight differing types were selected for more intensive comparison with previously described strains. The physical properties, transmission by *Myzus persicae,* and protective action on zinnia against Price's No. 6 strain was similar for all. Similar reactions occurred on 54 plant species, but seven species offered some means of differentiation. Five of the isolates were believed to be pure strains because they could not be separated into two or more component parts by any available means. The reaction on cowpeas, however, enabled the separation of a mixture of two types of strain. Nine strains previously described are accepted as valid. Other strains, differentiated largely on the geographical area or the host from which isolated, are to be regarded as doubtfully distinct. Five new strains (or substrains) were differentiated in the present study on the basis of minor variations. All the evidence emphasizes the remarkably constant character of the virus obtained from a great variety of sources.

Overwintering of the cucumber-mosaic virus. FAAN, HWEI-CHUNG, AND JAMES JOHNSON. A 3-year field survey was made in Wisconsin for natural hosts of the cucumber-mosaic virus, largely in the vicinities of commercial cucumber and tobacco fields and home gardens. Collections were all inoculated to tobacco as a test plant in the greenhouse. Inoculations from 635 collections involving 48 annual species and from 1362 collections including 62 biennial or perennial species yielded the cucumber-mosaic virus from 360 samples. Thirty-one species, including weeds and ornamentals, were infected, of which 16 were possible overwintering hosts. Seven perennial hosts previously reported were confirmed. Nine other perennial hosts were found, including common motherwort, flowering spurge, white cockle, and phlox. The overwintering hosts were mostly in or near home gardens in villages. Infected milkweed was not secured in or near 25 commercial cucumber fields or 21 tobacco fields. Cucumber mosaic in such fields could not be definitely associated with overwintering hosts in the vicinity. Unless insect vectors move over relatively long distances from home gardens in villages, it appears that the origin of the disease in commercial fields cannot be accounted for in Wisconsin through milkweed or other known overwintering hosts.

Soil and tree acidity in relation to susceptibility to Dutch elm disease. FELDMAN, A. W., AND N. E. CAROSELLI. A method for measuring the pH of the outer xylem tissue of elms has been developed. The alkalinity of greenhouse-grown potted elm seedlings increased after pH 5.5 soil was limed to pH 7.2–7.4. Within 7 days the pH of roots and stems reached 6.9 and 7.2 respectively and then became rapidly lower than the values for seedlings in nonlimed soil. In spite of vascular tissue discoloration, no wilt developed in limed inoculated trees. All nonlimed trees wilted within 21 days after inoculation. Reinoculation of seedlings 25 days after soil treatment with lime resulted in 40 per cent mortality. Results from woodlot elms receiving lime or lime plus therapeutant, though incomplete, show similar trends. Change in pH does not directly account for the greater pathogen establishment in seedling elms exposed to 5-day dark treatment prior to inoculation with *Ceratostomella ulmi*, since acidity increase is only 0.2–0.3 pH even after 20 days of darkness. Delay of wilt symptoms from 3 to 4 weeks by host absorption of hydroxyl-ions supports laboratory data on the inactivation of *C. ulmi* toxin complex at and above pH 7.0. Soluble carbohydrates are increased in elms grown in limed soil. Foliage and trunk absorption of sugars has retarded wilt symptoms for 3 to 4 weeks.

Fungicidal adhesives. FELIX, E. L. Tests in Tennessee indicate that natural and synthetic resin emulsions such as Nu-Film and Resyn Adhesives 3024 and 3605, at the rate of 1 pt. to 100 gal. of spray material, are exceptionally good stickers for fixed coppers and Bordeaux mixture and deserve wider usage, when residue is not a factor. While primarily sticking agents, they act also as spreaders. Dispersion of Resyn Adhesive 3605 is accelerated by its negative charge. Nu-Film and Resyn Adhesive 3605 are coagulated in solutions of copper and zinc sulphates; and the latter by hydrated lime. The old rule of supplements in Bordeaux mixture, therefore, should be followed: addition after preparation. Resyn Adhesive 3024 appears to be more compatible. An adhesive such as Armour's Sticker at the rate of 1½ lb. to 100 gal. of spray material or 10 per cent by weight of dust has proved generally useful. Bulk and wettability of the diluent (Attaclay vs. Pyrax) appeared to make no difference in sticking properties under the conditions of the tests.

Fungicidal control of leaf spot and mildew on nursery cherry trees in Iowa. FINK, H. C., AND O. F. HOBART, JR. Fungicides were applied to 1-year-old cherry trees in the nursery for comparison of leaf spot and powdery mildew control in 1948, 1949, and 1950. Fungicides and the rate in lb. per 100 gal. water were: ferbam, ½; zineb, 1, 2, and 4; Phygon, 0.5, 1, and 2; Bioquin, 0.5, 1, and 2; 341 B cherry spray, 1, 2, and 4; Puratized, 0.5, 1, and 2; Merck H258M, 0.75, 1.5, and 3; Ortho 406, 2; tribasic copper sulfate, 2, 4, and 8; lime sulfur, 2.5, and wettable sulfur, 4. An 8–4–100 Bordeaux mixture also was used. For leaf-spot control, as measured by percentage defoliation, Bordeaux mixture was most effective. Percentage defoliation of Bordeaux-sprayed trees was 2.0, 16.7, and 60.8 in 1948, 1949, and 1950, respectively. Tribasic copper sulfate (4–100) was nearly as effective. Percentage defoliation of tribasic-sprayed trees was 7.0, 21, and 72. For powdery mildew control, tribasic (4–100), Bordeaux, and Phygon (4–100) were most effective, in that order. Among the variously sprayed plots in 1949, tree height and caliper were greatest in those sprayed with tribasic, Bordeaux, and ferbam. Second-year growth was greatest in these same plots. All were uniformly sprayed with tribasic in 1950.

Comparative physiology and pathogenicity of eight isolates of Ceratostomella ulmi. FREDERICK, LAFAYETTE, AND F. L. HOWARD. Effect of pH on mycelial growth, toxin titer, and polysaccharide yield was investigated for eight culturally distinct isolates of

Ceratostomella ulmi. Shaken cultures in an unbuffered liquid medium (pH 5.2) and buffered pH levels (4.0 and 7.1) were used. Highest mycelial yields were produced by six isolates in the buffered pH 7.1 series, and by two in the buffered pH 4.0 series. A pH range of 5.2 to 6.5 appeared optimum for mycelial growth for all isolates in liquid media. All isolates produced highest titer toxin in the buffered pH 4.0 series. Highest polysaccharide yields were obtained in the unbuffered series, two produced a trace in the buffered pH 7.1 series, and none formed measurable amounts in the buffered pH 4.0 series. Two isolates produced no polysaccharide in any buffered series; yet one of these formed the most potent toxin. No correlation was found between mycelial growth and toxin titer or polysaccharide yield; nor between toxin titer and polysaccharide yield or degree of pathogenicity on seedling elms. In toxin-treated and inoculated seedlings alike, tyloses and gumlike substances formed in vessels; whereas unused medium and water were without effect. Intensity of vascular discoloration was not proportional to the wilting caused by the isolates. Leaf trace injury contributes materially to the symptom complex.

A Phytophthora root rot of tomatoes. FULTON, J. P., AND NEIL D. FULTON. A root rot of tomatoes caused by *Phytophthora parasitica* Dast. has been observed several times in Arkansas. In comparison of the cultural characteristics and parasitic activity, the pathogen obtained from tomato roots was indistinguishable from two other isolates of *P. parasitica* obtained from tomato fruits with typical buckeye-rot symptoms. In greenhouse inoculations, five varieties of tomatoes were equally susceptible. A marked reduction in activity of this pathogen was observed in unsterilized soil as compared with sterilized soil. In preparing inoculum, an agar medium using Austrian winter peas gave excellent sporangial development. Exposure of cultures to continuous illumination stimulated sporangial production. Aeration of cultures enhanced both the production of sporangia and the production of oospores.

The reinfection of mosaic leaves of Nicotiana sylvestris by other strains of tobacco-mosaic virus. FULTON, ROBERT W. Mosaic leaves of *Nicotiana sylvestris* infected with tobacco-mosaic virus were not completely immune to other strains of the virus. Strains which caused necrotic local lesions on healthy *N. sylvestris* leaves also produced such lesions on mosaic leaves. Reinfection occurred only with virus extracts more concentrated than about $1:10^3$, whereas lesions were produced on healthy leaves at dilutions of $1:10^6$. On mosaic leaves the lesions were slower to appear, and remained smaller than on healthy leaves. Only the dark-green areas of mosaic leaves were susceptible to reinfection. Such areas contained a lower concentration of virus than the light-green areas. The inoculation of halves of healthy leaves with mosaic-type strains resulted, in 4 days, in nearly complete immunity to necrotic-type strains in the inoculated half-leaves. The virus concentration in these half-leaves exceeded that in the dark-green areas of systemically infected mosaic leaves. When mosaic leaves were inoculated with mosaic-type strains, no additional resistance to necrotic-type strains was induced. It was concluded that necrotic-type strains were able to enter and multiply in cells containing a low concentration of another strain of the same virus.

Effect of seed treatment on forage legumes in wet and dry soil. GERDEMANN, J. W. The effect of three chemical seed treatments on red clover, alfalfa, and sweet clover was studied in wet and dry soil. Red clover seed treated with three different rates of Arasan, Ceresan M, and Phygon and planted in wet and dry soil showed no significant increase in emergence. Some treatments which appeared harmless in wet soil reduced the emergence or injured the seedlings when the seed was planted in dry soil. Alfalfa was neither benefited nor injured by most treatments. The reaction to treatment was nearly the same in wet and dry soil. One per cent Ceresan M caused injury to seedlings in wet and dry soil in the greenhouse, and 1 per cent Phygon reduced the emergence in the field under drouth conditions. Sweet-clover emergence was increased by treatment with Arasan and lower rates of Ceresan M in the greenhouse. Another seed lot was not benefited by any seed treatment. A number of treatments which were harmless in wet soil reduced the emergence or injured the seedlings in dry soil. Arasan, which appeared beneficial or harmless in dry soil in the greenhouse, significantly reduced the emergence of sweet clover in the field under drouth conditions.

Pelleting soybean seed with fungicides for control of damping-off and root rot. GIBLER, JOHN W. In a preliminary test for controlling damping-off, Ottawa Mandarin soybean seeds were pelleted with 33 different fungicides and planted in the greenhouse in soil artificially infested with *Rhizoctonia solani.* Arasan, Arasan SF, Tersan, and Leafox 200A were very effective in the preliminary test; the remainder were either phytotoxic or ineffective. The former were further tested in the greenhouse and field at the

rates of 8, 16, 32, 48, and 80 oz. of fungicide pelleted on 100 lb. of seed along with a standard dust application of 3¼ oz. of fungicide per 100 lb. of seed. The dust application did not control damping-off and root rot, but control was excellent with the 8-oz. application pelleted on the seed. This rate was as effective as the higher rates.

Etiology and host range of X-disease of chokecherry. GILMER, R. M. Etiology and host range of certain of the Wisconsin strains of the X-disease virus were studied in both field and greenhouse. The virus was recovered from both roots and aerial portions of dormant infected chokecherry, provided sufficient time had elapsed from the date of inoculation to permit the virus to move throughout the entire host. Movement of the virus from the site of inoculation was relatively slow. Large 8-year-old chokecherry trees failed to show complete involvement of aerial branches in symptom expression as late as 23 months from time of inoculation. The virus was not recovered from symptomless branches 11 months after inoculation if these branches were at least 12 to 18 in. away from portions of the trees which were expressing symptoms. At least four strains of the X-virus, based on symptomatology, virulence, and ease of transmission, were discovered. New hosts of the virus in the genus *Prunus* include: *P. domestica* var. Italian Prune, *P. pennsylvanica*, *P. maackii*, *P. padus*, *P. glandulosa*, *P. pumila*, *P. dunbari*, *P. maritima*, and *P. tomentosa*. *Prunus domestica* var. Italian Prune and *P. pennsylvanica* are symptomless carriers of the virus. *P. serotina* is considered immune.

Lipolytic activity of certain fungi important in grain deterioration. GOODMAN, J. J. Ground corn, with and without the original corn oil, was inoculated with *Penicillium solitum*, *Aspergillus flavus*, and *A. amstelodami*, three species important in deterioration of stored grain. The fat acidity (benzene extract) and water acidity were tested during 25 days. Fat acidity of the corn containing oil rose initially and then decreased, whereas that of the corn without oil remained low. Water acidity varied with the species but not with the substrate. Fat acidity was derived almost exclusively from corn oils rather than from metabolic acids of the fungi. The fungi utilized the constituent acids of corn oil as the sole carbon source. Mycelial preparations of these fungi contained active lipase. *P. solitum* and *A. flavus* had much higher lipase activity when grown on corn oil than when grown on sucrose as a carbon source. *A. terreus* and *A. candidus* contained much less lipase and the amount produced did not vary with the carbon source. *A. amstelodami* contained active lipase when grown on sucrose and increased the fat acidity of corn considerably, yet generally failed to utilize corn oil as a carbon source, though it utilized, to some extent, both fatty acids and glycerol.

Overwintering and association of three bacterial pathogens of soybean. GRAHAM, J. H. Three soybean leaf-spot pathogens, *Xanthomonas phaseoli* var. *sojensis*, *Pseudomonas glycinea*, and *Pseudomonas tabaci*, which cause bacterial pustule, bacterial blight, and wildfire, respectively, were found to be seed-borne and to overwinter in diseased leaves in North Carolina. Overwintering on seed is probably the more significant means of survival. A close association between *X. phaseoli* var. *sojensis* and *P. tabaci* was noted in the sequence of disease development; *X. phaseoli* var. *sojensis* appears first, followed by *P. tabaci* on the diseased leaves. In greenhouse and field studies, *P. tabaci* infected soybean leaves primarily through bacterial pustule lesions. In laboratory tests *P. tabaci* killed either of the other two organisms when incubated in broth media. Further studies showed that *P. tabaci* produced a diffusible substance in culture antagonistic to *X. phaseoli* var. *sojensis* and *P. glycinea*.

Nematode injury to tobacco, cotton, and corn in relation to populations of root-knot and meadow nematodes. GRAHAM, T. W., AND Q. L. HOLDEMAN. Root knot, caused by *Meloidogyne* spp. and nematode root rot caused by *Pratylenchus* spp. were studied on tobacco, cotton, and corn. Root injury (disease index) was recorded at biweekly intervals during the growing seasons of 1949 and 1950. Nematode populations were counted from uniform weighed root samples. Greenhouse soil heavily infested with root knot was added to field row plots. Separate soil lots infested with meadow nematodes were added to root-rot plots. Other plots so treated were fumigated with DD mixture and ethylene dibromide. Maximum root knot on tobacco and cotton occurred in late August and early September. Meloidogyne populations increased slowly during the season and decreased only after severe root decay had started. Root knot failed to develop significantly on corn. Root-knot damage and Meloidogyne populations were greatly reduced by fumigation. Root rot increased regularly on all crops and Pratylenchus populations reached a sharp peak, then decreased rapidly on tobacco and corn before maximum root decay occurred. Population counts on cotton were erratic. Fumigation reduced root rot less than root knot. Pratylenchus population peaks were 1 to 2 weeks later than in unfumigated plots.

Inhibition of the potato-scab organism by soluble aluminum ions. GRIES, GEORGE A. Several strains of *Streptomyces* (*Actinomyces*) *scabies* which grow well in buffered nutrient solution at pH values as low as 4.2–4.6 have been isolated from tubers grown on mineral and muck soils in Indiana. The addition of 20 p.p.m. or more of aluminum ions as $Al_2(SO_4)_3 \cdot 18\ H_2O$ to the culture solution inhibits the growth of these strains below pH 5.0–5.2. Magnesium ions have no ability to antidote this aluminum toxicity. Above pH 5.2, aluminum is so insoluble that its presence is not inhibitive. It is suggested that the successful control of potato scab by soil acidification is in part due to the effect on solubility of aluminum. Muck soils in which scab developed at low pH values were found to be low in active aluminum. The addition of aluminum to organic soils for the control of scab does not appear to be practicable.

Developmental cytology of Puccinia graminis tritici races 56 and 15B on seedlings of Hope and Triticum timopheevi. GRIMM, GORDON R., AND JAMES G. DICKSON. Ten-day-old wheat seedlings were grown and inoculated at 16° and 28° C. during the winter. Macroscopic uredial development at various combinations of temperature, time, host, and race of pathogen was coordinated with the cellular reactions of both host and pathogen. Material for microscopic examination was killed in Karpochenko's fixative and stained using Conant's Triarch quadruple staining technique. Race 15B on Hope and *Triticum timopheevi* was characterized by 3+ and 3 – infection types, respectively. Temperature influenced rate of development more than infection type. Cytological observations showed a generally compatible reaction between host and mycelial cells, especially at low temperature. Race 56 on Hope produced an infection type 2 at 16° C. and a type 3 at 28° C., accompanied by more chlorosis at both temperatures than was evident with race 15B. A semicongenial relationship between host and mycelium was established only after an initial hypersensitive reaction which was evident in 3- and 5-day-old infections at 28° and 16° C., respectively. High resistance (type 0;) of *T. timopheevi* to race 56 was evident at both temperatures. Cytological observations showed a complete incompatibility between host and pathogen following the development of mycelium from the substomatal vesicle.

The reaction of Perfection-type peas to Wisconsin bean virus 2 isolates from pea. HAGEDORN, D. J. Perfection peas commonly have been thought resistant to bean virus 2. Considering that these peas are widely grown in Wisconsin and that this bean virus is easily isolated from mosaic-diseased peas in this State, studies were made to test the reaction of Perfection peas to four bean virus 2 isolates from pea. Thirty-six varieties were studied in the greenhouse. Using mechanical inoculation, each variety was tested six times with each virus isolate. About 240 plants of each variety were studied—about 60 with each of the four isolates. Most varieties tested, including Wisconsin Perfection, were resistant, but eight were susceptible to at least one bean virus 2 isolate. Four of the susceptible varieties were rarely infected. The other four were susceptible to all four virus isolates. These results may help explain why bean virus 2 is so readily obtained from mosaic-diseased peas in Wisconsin, and may indicate that Perfection type peas are not so resistant to this virus as had been assumed earlier.

The relationship between Wisconsin pea stunt and red-clover vein mosaic. HAGEDORN, D. J., AND E. W. HANSON. Comparative studies of the viruses causing Wisconsin pea stunt and red-clover vein mosaic were made because the symptoms produced by these two viruses on red clover and on pea were strikingly similar. Comparisons of symptoms, temperature relations, host range, the varietal reactions of 10 varieties of peas, and physical properties were made and in all these respects the viruses were found to be essentially the same. In the host-range study 15 species from three families were tested. It is concluded that the virus causing Wisconsin pea stunt and the virus causing red-clover vein mosaic are the same. Both of these diseases are widespread in Wisconsin and are frequently found in close proximity.

A leaf spot of tomato caused by a new Stemphylium sp. HANNON, C. I., AND G. F. WEBER. A circular to irregular leaf spot, 1–4 mm. in diameter, of watery-gray color and slightly sunken when young, dry and brown with age, sometimes surrounded by a light chlorotic halo, appeared naturally on tomato plants and caused defoliation. These lesions yielded an organism of Stemphylium type probably distinct from described species. The organism grew and sporulated well on potato-dextrose agar at 15°–26° C., and between 20° and 32° C. produced a characteristic amber to burgundy pigmentation. Cultures made from sterile tufts of pink mycelium produced at 26°–35° C. yielded sterile cultures. Conidiophores are long, seldom branched, with swollen terminal cells upon which the conidia are borne singly. Conidiophores continue growing through the terminal swelling after production of conidia, resulting in a series of swellings. Conidia are muriform,

brown, smooth, with 1 to 6 (average 2–3) deeply constricted cross septa; longitudinal septa vary with age, none to many; conidia 28–55μ × 11–23μ. Light is necessary for production of conidia. Seedlings of 10 tomato varieties were inoculated and lesions similar to the originals appeared on all varieties within 72 hr. after removal of plants from moist chamber. The reisolated fungus coincided with the original isolate.

Resistance to nematodes in pepper. HARE, W. W. A collection of 162 lines of pepper (*Capsicum frutescens* L.) was tested for response to nematodes, species undetermined, which incited gall formation and destruction of roots. Tests were conducted in infested soil in a greenhouse bench. Of four trials made on different dates, the first two each consisted of half the collection, each line being replicated four times; the third and fourth trials contained 26 resistant and 10 susceptible lines selected from earlier trials. The 36 lines were arranged in a lattice design with six replications in both cases. In making evaluations, the plants of each lot were placed individually in one of five arbitrary classes, described as healthy, slightly, moderately, or severely diseased, and dead, on the basis of number and size of galls and amount of root destruction. Indices were calculated from this classification. Highly significant differences between lines in individual trials and consistency of rank in different trials showed a high degree of resistance to gall formation and root destruction in certain of the lines. Named varieties in the most resistant material included Santanka, Anaheim Chile, and Italian Pickling. Commercial varieties of sweet pepper were among the most susceptible lines.

Breeding tomatoes for disease resistance. HARRISON, A. L. Commercial type tomatoes with resistance to Fusarium wilt, Alternaria collar rot, and root-knot have been developed by using a combined inoculation technique. Roots and stems of tomato seedlings with three to four leaves are immersed in a combined suspension of *Fusarium oxysporum* f. *lycopersici* Snyder & Hansen and *Alternaria solani* (Ell. & G. Martin) Sor. and then set immediately in soil in the greenhouse. Lines that are susceptible to wilt but not collar rot die from wilt, and lines susceptible to collar rot but not wilt die from collar rot. Only those plants that are resistant to both diseases survive this combined inoculation if the environment is favorable for disease development. In some tests the tomato plants are grown in soil thoroughly infested with *Heterodera marioni* (Cornu) Goodey to check on resistance to root-knot. In such tests only individuals with high degree of resistance to all three diseases are set in the field for further observation. Additional observations are made in the field for resistance to leaf mold (*Cladosporium fulvum* Cke.), and gray spot (*Stemphylium solani* Weber). At the present time breeding lines with commercial-size fruits have been developed with resistance to one or more of the following diseases: Fusarium wilt, collar-rot phase of early blight, root knot, gray spot, and leaf mold.

Growth of diseased plant tissue in vitro influenced by the concentration of organic acids. HILDEBRANDT, ALBERT C. Studies of the metabolism of host tissue in diseased growth has provided a clarified view of the influence from basic nutrients. Such diseased host tissue has been grown *in vitro* under closely controlled conditions. These methods offer important possibilities for study of the fundamentals in various plant disease relationships. Bacteria-free tissues of crown-gall origin from marigold, Paris daisy, periwinkle, and sunflower and tissue from a hybrid tobacco were incubated *in vitro* on synthetic media. None of the species increased in weight on media without sucrose but containing, respectively, different concentrations (4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03, or 0.015 per cent) of salts of each of the following: acetic, citric, formic, fumaric, glycolic, gluconic, glutaric, lactic, malic, malonic, oxalic, propionic, pyruvic, stearic, succinic, or tartaric acids. However, with 2 per cent sucrose the concentrations of the salts of organic acids was a critical item in determining the amount of growth that appeared. On the 16 salts some species grew well, some grew poorly, and some failed completely. Certain species grew to varying degrees over a wide range of concentrations; others grew only on a narrow range of concentrations.

Investigations on a cucumber virus mechanically transmitted from sour cherry. HOBBS, GORDON A. A number of cucurbits of foreign introduction were tested for susceptibility to a virus mechanically transmitted to cucumber from sour-cherry leaves having symptoms of necrotic ring spot. Forty-six of the 47 cucumber varieties and four of nine pumpkin varieties were susceptible but none of the 12 squash or 7 watermelon varieties tested became infected. When compared on 9 cucumber varieties, 9 regional isolates of the cherry virus differed in 1) apparent concentration as expressed by number of rings developed on cotyledons of the test plants, 2) symptoms produced, and 3) temperature requirements. Light intensity did not seem to be critical for infection or symptom development; day length was not tested. From an experiment involving a series of transfers from yellow portions of infected cucumber leaves it appeared that the virus is

more concentrated in the yellow portions of the developing mottle. All attempts to reinfect cherry with the virus from cucumber by patch grafting and mechanical techniques failed.

Tobacco stem rot of transplants and barn rot caused by Pythium aphanidermatum. HOLDEMAN, Q. L. A Pythium stem rot of tobacco transplants, previously reported in Sumatra and in Connecticut, was observed in South Carolina in 1950. A soft rot occurred just below the surface of the soil. Resets placed adjacent to the dead plants frequently became diseased. This necessitated frequent resetting, which resulted in an uneven stand of plants. Barn rot, a Pythium soft rot of ripe leaves that developed during early stages of curing, was most severe on bottom leaves harvested during wet weather. The rot spread rapidly along the midrib and into the leaf lamina, causing a brown to brownish-black discoloration. Infected parts of the leaf produced worthless tobacco. *Pythium aphanidermatum* (Eds.) Fitz. was the causative organism for both stem rot and barn rot.

Stimulation of melanogenesis in Streptomyces scabies. HOLLIS, JOHN P. The natural correlation between melanin production and pathogenicity in *Streptomyces scabies* (Thaxt.) Waks. & Henr. served as an opening wedge to a study, now in progress, of biochemical characteristics in relation to pathogenicity. It was desirable, at the outset, to develop a melanin-indicator medium to enable separation of melanogenic and nonmelanogenic colonies of *S. scabies* within a short culture period. Among a variety of enrichment substances tested, yeast autolysates and vitamin-free casein hydrolysates were markedly stimulatory. While auxanographic tests with 22 amino acids indicated some slight stimulation of melanogenesis, single addition and omission tests with 10 water soluble vitamins were negative. Results with simulated casein hydrolysates were consistent with the view that stimulation of melanogenesis is attributable to a balanced amino acid-peptide complex.

Indications of a New-World origin of tobacco-mosaic virus. HOLMES, FRANCIS O. A study of solanaceous plants, especially in the genus *Nicotiana*, has disclosed evidence supporting the hypothesis that tobacco-mosaic virus has a New-World origin. Areas characterized by relatively resistant species and varieties are centrally located within larger areas characterized by closely allied but highly susceptible species. Tobacco-mosaic virus is now essentially world-wide in distribution, probably as a result of diffusion of tobacco products by commerce. It seems probable that its distribution was more limited formerly and that its long-continued presence in the areas now characterized by relatively resistant host plants was responsible for reduction of competitive vigor in susceptible species there, with resultant encouragement of resistant mutants.

The corn embryo as a factor in resistance in Pythium during germination. HOOKER, ARTHUR L. Investigations on the nature of resistance to Pythium seed decay in corn indicated the embryo was either resistant or susceptible when grown independently of the other kernel parts. Excised embryos were placed on a young culture of *Pythium debaryanum* on potato agar and covered with moist filter paper. After 4 days' incubation at 12° C., the embryos were removed, surface-sterilized (2 min. in mercuric chloride, 1: 1000), washed in sterile distilled water, and replated on a synthetic medium for growth of embryos *in vitro*. Following subsequent incubation at room temperature, embryos from resistant inbreds had few signs of infection and developed normally. Conversely, embryos from susceptible inbreds manifested various stages of decay and many were killed. In resistant inbreds, excised dormant embryos from steeped kernels were susceptible but became resistant after 24 hours' development on moist filter paper. Similarly treated embryos from susceptible inbreds remained susceptible. Evidence from low-temperature studies (11° C.) in Pythium-infested soil and from pure culture inoculations of excised embryos indicated that resistance developed in the embryos germinating at both high and low temperatures. In resistant inbreds this resistance was attained earlier than in susceptible inbreds.

Potato tuber growth and scab infection. HOOKER, W. J., AND O. T. PAGE. Cobbler and Ontario potatoes were grown in an inverted 8-in. flower pot with a hole cut in the side for observation of tuber growth and scab development. Scab inoculum was sprayed on small tubers during growth. The pattern of periderm expansion was determined by painting small tubers with India ink. In Cobbler, after exposure to inoculum, deep scab was first evident as necrotic spots which generally developed around the expanding apex of the tuber. Subsequent development resulted in rapid enlargement of the apical portion and retarded enlargement of the basal portion of the tuber. The necrotic spots coalesced during continued tuber growth and formed a more or less continuous ring

between the newly expanded, apparently healthy apex and the inked basal portion of the tuber. Although radial growth of the basal portion was evident by the expansion of the India ink pattern, infection in this area was rare. The center of rapidly growing tuber tips and eyes remained free of visible infection. Growth in Ontario, a resistant variety, generally was not accompanied by rapid apical growth. The shallow scab that developed after exposure to inoculum was not clearly associated with localized areas of expansion.

A new technic for incubating seed corn in cold soil for disease tests. HOPPE, PAUL E. A method has been devised which greatly reduces the refrigeration space required for conducting low-temperature incubation tests with seed corn. The seed is incubated in a small volume of wet soil in a glass tumbler for 8 or more days at the desired temperature. After incubation the kernels are removed from the tumbler and replanted in a warm greenhouse, where data are taken at the 4-leaf stage of seedling growth. With the reduced soil volume an ordinary electric refrigerator is adequate for incubating many samples at the same time. The usefulness of the technic for evaluating different seed-treatment dusts was indicated when identical results were obtained with it and with the large walk-in refrigerator previously used. In another experiment with the "glass-tumbler" method, ultimate disease effects were compared after untreated and Arasan-dusted, crown-wounded kernels had been incubated at 4° C. and 11° C. for 5-, 10-, and 15-day periods in peat soil and in fungus-free sand. High levels of disease resulted only from incubations of untreated seed in peat soil. Here seed decay reached 100 per cent for incubations of 10 or more days, at either temperature. Disease level was higher at 11° C. than at 4° C. following 5-day incubations. The results indicate that low temperature in itself was not injurious but served merely to predispose unprotected, slowly germinating kernels to attack by soil fungi.

A new anthracnose of vetch. HORN, N. L., AND J. G. ATKINS, JR. A species of *Colletotrichum* was consistently associated with and isolated from diseased common vetch, *Vicia sativa*, collected from several different areas of Louisiana during the late winter and spring months of 1948 and 1949. The anthracnose was more severe in southern Louisiana where the plants were completely defoliated and killed. The first symptoms of the disease occurred as leaf and stem spots, both of which became sufficiently numerous to cause leaf blight and stem blackening. Hairy vetch, *V. villosa*, was resistant in field tests where common vetch was killed. In greenhouse inoculation tests, common vetch plants were killed within 7 days. On the basis of greenhouse pathogenicity tests, *V. villosa* and *V. atropurpurea* were fairly resistant; *V. sativa*, *V. articulata*, *V. pannonica*, *V. angustifolia*, *V. dasycarpa*, and *V. grandiflora* were susceptible. Austrian Winter peas were moderately susceptible in greenhouse pathogenicity tests, as well as in nursery plots adjacent to severely diseased common vetch. On the basis of pathogenicity tests and morphological and cultural studies the fungus is believed to be a species of *Colletotrichum* different from any reported attacking vetch.

Oxidized substituents decrease fungitoxicity of reduced compounds. HORSFALL, JAMES G., AND SAUL RICH. Numerous fungus toxicants depend upon reduced groupings like NH_2, OH, and –S–S–. Frequently the fungitoxic action of such compounds on spores may be quenched when oxidized groupings are attached to the same molecule; i.e., sulfonation of 8-quinolinol and bis(4-hydroxyphenyl) isopropane; adding a sulfone grouping to phenylene diamine; carboxylation or nitration of diphenyl disulfide; and adding a C=O grouping to benzidine, phenyl hydrazine, 3-hydroxyphenanthrene. Assuming that the Krebs cycle is involved in the metabolism of the spores of the test organisms, it is possible that the reduced compounds act to prevent the oxidation of the carboxylic acids in that cycle and thus to rob the spore of energy. Apparently the effect of the reduced substituent on an essential carboxylic acid can be antidoted by a carboxylic acid unit or a sulfonic acid analogue on the same molecule or even on a different molecule. The effect is much more pronounced for *Sclerotinia fructicola* than for *Stemphylium sarcinaeforme*.

Indexing potatoes for virus X, including the use of tuber juice. HOYMAN, WM. G. A total of 1,378 plants of Bliss Triumph, White Rose, and Cobbler were indexed on 36 varieties of *Capsicum annuum*. Of these, 98 were negative, but further testing indicated they were infected with virus X. Considerable difference in susceptibility and expression of symptoms was observed among the varieties of peppers. In some instances the only symptoms were pimples on the stems. *C. annuum* is not considered a reliable indicator plant for virus X. *Gomphrena globosa* was more satisfactory as an indicator for this virus. Juice from potato leaves was used; a rapid and convenient method was developed by rubbing freshly cut tubers on the leaves. Number 400 carborundum was dispersed on leaves of indicator plants with a No. 119 DeVilbiss powder blower. Of 3,763 tubers of Pontiac, Bliss Triumph, Cobbler, and Green Mountain tested, 19 gave no reaction.

From a second test of the 19 negative tubers, 17 reacted, and the remaining two were Cobblers that gave no reaction when tested the third time. Juice from the leaves of plants grown from these two tubers gave a positive test on *G. globosa*. Some tubers of Green Mountain were carrying a weak strain of virus X.

Stem rot of peanuts. JACOWAY, T. H., AND J. H. OWEN. A Diplodia stem rot of peanuts has been observed in the Georgia-Florida area causing up to 30 per cent infection in some fields. Morphologically and physiologically the organism is identical with *Diplodia natalensis* Pole-Evans. The perfect stage has not been produced in culture nor found on peanuts in the field. Mature conidia measure 11–16 $\mu \times$ 19–30 μ. Immature conidia are hyaline and one-celled, while the mature spores are brown and two-celled. On potato-dextrose agar the cardinal temperatures are 15°–31°–40° C. Infection may occur anywhere on a runner or the central stem. The invaded tissues are browned in early stages, later becoming black and necrotic. Pycnidia have been observed only on the stems. The peanut *Diplodia* is pathogenic on *Citrullus vulgaris*, *Citrus sinensis*, and *Ipomoea batatas*. Infection resulted when field-plot peanuts were wounded and inoculated with spore suspensions and mycelium.

Foot rot of Chinese hibiscus. JAMES, M. O., AND A. G. PLAKIDAS. A fatal disease of the Chinese hibiscus, *Hibiscus rosa-sinensis* L., has appeared in various localities in Louisiana. The causal organism has been tentatively identified as *Phytophthora cactorum* var. *applanata* Chester. The disease is characterized by decay of the crown and main roots of the plant, accompanied by gradual wilting and yellowing of the foliage and discoloration of the stem. Foot rot occurs naturally on the Kona, Brillantissima, Venus, Hawaiian Fountain, Agnes Gault, Lambert, Pink Beauty, Jigora, Dolores, Euterpe, Peachblow, and Minerva varieties. Under lath-house conditions, the disease was produced on the Kona, Euterpe, Minerva, Psyche, Peachblow, and Brillantissima varieties by artificially inoculating steam-sterilized soil, under poor and good drainage conditions, with pure cultures of *P. cactorum applanata*.

A new disease of dill. JANSON, B. F. For the past two years a destructive root rot and wilt of dill (*Anethum graveolens* L.) has been present in Ohio where dill has grown in the same soil for several years. In some fields the loss was 100 per cent. Plants are susceptible at all ages, but young seedlings are most susceptible. Infected seedlings suddenly wilt and die. In older plants there is gradual yellowing of the oldest leaves, extending upward into the successively younger leaves until the plant dies. The vascular system of infected plants becomes dark and the roots decay. A species of *Fusarium* was consistently isolated from the roots and lower stem of diseased plants. The pathogenicity of monoconidial isolates varied from 2 to 100 per cent. Several species closely related to dill and other species known to be susceptible to Fusaria were not susceptible to this organism. Steam sterilization of infested soil taken from the fields and from the greenhouse successfully controlled the disease, but seed treatment with various commercial compounds did not. A certain degree of resistance is apparently present in some dill plants.

Soybean seed treatment tests, 1947–1950. JOHNSON, HOWARD W. A test comparing Arasan and Arasan SF on seed of five soybean varieties was planted by cooperators at ten locations in the South in 1947. The two treatments resulted in statistically significant stand increases of approximately 10 per cent at all locations except one, on each of three planting dates. At Stoneville, Mississippi, in 1948, Dow 9-B, Ceresan M, New Improved Ceresan, 2 per cent Ceresan, Spergon, and Arasan on seed of eight soybean varieties resulted in highly significant stand increases ranging from 7 to 15 per cent; yield differences were not significant. At Stoneville in 1949, improvement in stand over the nontreated checks ranged from 4 per cent with Dow 9-B to 26 per cent with Spergon in a test involving ten treatments and four seed lots. Yield increases due to treatment with New Improved Ceresan, Spergon, and Arasan were 3.7, 4.1, and 4.5 bu. per acre, respectively; all significant statistically. At Stoneville in 1950, percentage improvement in stand over the nontreated checks was 12 for Vancide 51, 13 for Arasan, and 16 for Spergon in a test involving 16 soybean strains. It appears that seed treatment will consistently improve soybean stands in the South and in some years will increase yields.

Gene pairs conditioning pathogenicity in Venturia inaequalis. KEITT, G. W., AND D. M. BOONE. Six monoascosporic isolates of *Venturia inaequalis* were crossed in various combinations to study inheritance of pathogenicity as indicated by the lesion/fleck reaction with certain differential apple varieties. Four of these isolates incited flecks with the varieties Haralson, McIntosh, and Yellow Transparent. The remaining two

incited flecks with Yellow Transparent and lesions with Haralson and McIntosh. All eight ascosporic isolates from each ascus tested from crosses among members of the first group incited flecks with each of the three varieties. Similar progenies from crosses between members of the two groups showed a 4:4 segregation ratio for the lesion/fleck reaction with the varieties Haralson and McIntosh; however, with Yellow Transparent, 4:4, 2:6, and 0:8 ratios were obtained. For one of the latter crosses tests that included the additional variety, Red Astrachan, indicated that three independent gene pairs were concerned with inheritance of pathogenicity. One gene pair conditioned the lesion/fleck reaction with Haralson, the second with McIntosh and Yellow Transparent, and the third with Red Astrachan and Yellow Transparent. Since genes conditioning pathogenicity to Yellow Transparent were at two loci, a study of their interaction was possible. The flecking genes appeared to be epistatic to the lesioning genes.

Influence of light on the germination of sporangia of Physoderma zeae-maydis. KELMAN, ARTHUR, AND T. T. HEBERT. Light intensity, period of illumination, and quality of light influenced the germination of sporangia of *Physoderma zeae-maydis* in water suspensions. At 28° C. germination increased according to the logarithm of the light intensity as intensity was increased from 0 to 12 foot-candles. Further increase in light intensity to 393 foot-candles did not significantly increase germination. Light intensity requirements were less at 28° C. than at lower temperatures. Exposure of sporangia for alternating 12-hr. periods of light and darkness for 3 days was as effective as continuous illumination. If sporangial suspensions were placed initially in darkness for 24 hr., one 12-hr. period of illumination was sufficient for high germination. Differences in germination were obtained under different wave lengths of light. High germination occurred in blue, low germination in green, and no germination in yellow or red light. These differences in germination were attributed to quality of light rather than the quantity of energy received, since no germination occurred in red or yellow light even with high light intensities.

The influence of temperature upon the incidence of Rhizoctonia root rot of Lima beans. KENDRICK, J. B., JR. Biweekly plantings of Fordhook Concentrated Lima beans were made in two bean-growing districts in southern California to determine what differences, if any, existed in the amounts of root infection caused by *Rhizoctonia solani* Kuehn in early, mid-, and late-season crops. The plantings covered a period of 16 weeks from April through July. Incidence of Rhizoctonia infection was recorded biweekly, and a thermograph recorded both soil and air temperatures throughout the study. Plantings made so that mean soil temperature was 65°–70° F. at 6-in. depth for the initial 12 weeks had less Rhizoctonia infection than those planted later when soil temperature for a similar period was above 70° F. Optimum temperature for growth in culture of Rhizoctonia isolates from various districts in California varied from 64° to 91° F.; optimum temperature for disease development with these isolates varied from 59° to 95° F. It is, therefore, unsafe to state categorically that any particular season or soil temperature is most favorable for the development of Rhizoctonia root rot until the individual areas have been thoroughly studied with regard to strains of the Rhizoctonia and their growth habits.

Source and seasonal spread of certain viruses in peppers in southern Cailfornia. KENDRICK, J. B., JR., L. D. ANDERSON, AND R. C. DICKSON. Virus diseases are responsible for an annual moderate to heavy loss in production of sweet and chili peppers in southern California. A 2-year survey shows that tobacco-mosaic virus 1 and related strains and spotted wilt are the principal viruses causing damage. Forty-nine of 63 specimens, representing all symptom types observed during 1950, yielded tobacco mosaic or spotted wilt or both upon inoculation to *Nicotiana glutinosa*. Curly top and several unidentified viruses completed the list. Cucumber-mosaic strains apparently play a minor role in the pepper-virus complex. Data indicate that the viruses are often introduced into field plantings through contaminated transplant material. However, the greatest spread of the viruses seems to be associated with insect populations after the plants are set in the field. Good correlation exists between the incidence and density of *Myzus persicae* populations and the spread of the tobacco mosaics; and between populations of *Thrips tabaci* and *Frankliniella* sp. and the spread of spotted wilt. Early-season transplants seem to be subject to more losses by virus diseases than crops seeded directly. The principal weed host sources of these viruses are *Nicotiana glauca, Datura stramonium, D. meteloides,* and *Solanum nigrum.*

The acid test for phony disease of peach and its diagnostic value. KENKNIGHT, GLENN. Hutchins used dilute HCl in methyl alcohol as a test reagent for phony disease of peach, but only as an aid in confirming diagnosis. In this study the test was applied to two roots each of 191 apparently normal 3-year-old Elberta trees exposed to natural

infection. Ten gave a reading of six or more dots; 10, dots but necrosis; 11, red ring; one, two dots; 14, other uneven coloring; 145 were clear negatives. All surviving trees whose roots, in the absence of visible necrosis, showed a color reaction of two or more dots not centrally or concentrically located became phony. Other acids, organic and inorganic, produced the reaction. Liquid acids were effective alone, but with strong acids a diluent was desirable. Crystalline acids were dissolved in alcohols, acetone, or benzene. Reaction time varied from seconds to days depending upon the reagent and other factors. With organic acids, addition of $ZnCl_2$, KBr, or NaCl hastened the reaction. Water slowed it. Trichloroacetic acid in methyl alcohol was a particularly good reagent.

Seed treatment of alfalfa, red clover, and sweet clover. KERNKAMP, M. F. Alfalfa, red clover, and sweet clover seed were treated with Spergon, Spergon W, Arasan, Arasan SF, Phygon, Phygon XL, Phygon Paste, Tersan, Parzate, Fermate, and Dow 9B. Spergon and Arasan formulations were the best of the fungicides tested; the remainder were unsatisfactory. Response of these seeds to treatments is a function of environmental factors and seed quality. In environments unfavorable for germination or with seed of poor quality there was great response to seed treatment, whereas with excellent seed and unfavorable environments there was no response to seed treatment. Spergon and Arasan increased stands of alfalfa, red clover, and sweet clover in the greenhouse when seed was mechanically injured, planted in soil infested with *Rhizoctonia solani*, or planted abnormally deep. Seed lots of alfalfa, red clover and sweet clover from different sources were treated with Spergon and Arasan (8 oz. per 100 lb. seed) in greenhouse and field trials. The fungicides were applied by dusting and pelleting. Some seed lots responded exceedingly well to these treatments, whereas others did not respond. Averages of all treated seed lots of alfalfa and red clover were significantly better than the average of the controls. Results from field tests with sweet clover were so erratic that no conclusions could be reached. Dusting and pelleting were equally effective.

Resistance in soybeans to root rot caused by Rhizoctonia solani. KERNKAMP, M. F., AND JOHN W. GIBLER. All varieties of soybeans recommended in Minnesota are susceptible to root rot caused by *Rhizoctonia solani*, but recent investigations indicate that resistance to this disease is available by reselection within certain varieties or strains from hybrid origin. In routine tests of breeding material for resistance to *R. solani* some plants survived. Progenies from these plants were reselected in two subsequent field trials and were tested for resistance in three greenhouse trials. In each trial they were planted in soil artificially infested with five virulent races of *R. solani*. Twelve lines with a high degree of resistance resulted from this reselection: five from (Lincoln × Richland) × Lincoln; two from Mandarin × Richland; one from Mukden × Linman 533; one from Mukden × Wisconsin Manchu 3; one from Mukden × Richland; one from Ottawa Mandarin; and one from Flambeau.

Relative prevalence of fungi associated with root and crown rots of red clover in Wisconsin. KILPATRICK, R. A., AND E. W. HANSON. The relative prevalence of the various fungi associated with root and crown rots of red clover was determined at monthly intervals during the growing season at one location in 1949 and five locations in 1950. This was done for both first- and second-year stands. In both years, *Fusarium oxysporum*, *F. solani*, and *F. roseum* were most commonly isolated. *F. oxysporum* was easily the most prevalent fungus on first-year stands. In 1949 it constituted from 50 to 70 per cent of the total isolates; in 1950 it was less abundant but still the most prevalent. On second-year stands, its relative prevalence decreased but remained high. *F. solani* was more abundant in 1950 than in 1949 and was more prevalent on second-year stands. Its relative prevalence was low on very young plants but gradually increased. In general, *F. roseum* was also more prevalent on second-year stands. *Pythium* spp. constituted only a small percentage of the total isolates and were more commonly found on young plants. There was also considerable variation in the relative prevalence of such other fungi as *Gliocladium roseum*, *Rhizoctonia* spp., *F. moniliforme*, and *Phoma* sp.

Effect of copper sulphate on growth and fruiting of Coniothyrium fuckelii. KING, T. H., AND J. A. KEPLINGER. Some monosporous isolates of *Coniothyrium fuckelii* tolerated 6000 p.p.m. $CuSO_4$ in potato-dextrose agar. As the ability of the isolates to tolerate successively higher concentrations (500 to 6000 p.p.m.) increased, growth on potato-dextrose agar decreased when compared with the same isolate grown continuously on potato-dextrose agar. Some tolerant isolates failed to fruit in the first transfer back to potato-dextrose agar and a delay in fruiting occurred with other tolerant isolates. In either case, by four successive transfers back to potato-dextrose agar, ability to fruit and normal rapidity of pycnidial formation occurred. When isolates tolerant to 4000 p.p.m.

$CuSO_4$ were transferred back to potato-dextrose agar containing biotin or thiamin the first time, the normal delay of 6 days for fruiting was reduced to about 3 days. Also, isolates tolerant to $CuSO_4$ were less able to hydrolyze starch and gelatin than normal strains.

Toxicity of cycloheximide to certain wood-rotting fungi, and preliminary studies on absorption of the antibiotic by tissues. KLOMPARENS, WILLIAM. A biological assay technique was developed using *Poria microspora* growing on 2 per cent malt extract as the test fungus. Repeated tests showed that the method was accurate for determination of the presence of cycloheximide, an antibiotic produced by *Streptomyces griseus*, in concentrations as low as 0.5 p.p.m. The antibiotic suppressed fungal growth to a lesser degree in highly organic medium than in a synthetic broth. Although the cycloheximide was altered or removed from the medium by the growing culture, it was not recovered by an assay of the dried mycelium. Retention was also indicated by a 3-min. dip of 40 chips of *Tilia glabra* Vent. in a 200.0 p.p.m. solution, which when assayed showed that only 100.0 p.p.m. remained in the solution. Several wood-rotting fungi were grown in a medium containing 2.0 p.p.m. cycloheximide and were completely inhibited by this concentration. The fact that the antibiotic is retained, altered, or absorbed suggests that further work should be done to find an explanation of possible build-up in, and the nature of the action of, antibiotics in contact with organic living tissues.

Husk coverage and ear declination in relation to corn ear rots. KOEHLER, BENJAMIN. In a 3-year test with open-pollinated corn the following classes of ears were selected: 1) declined lower than horizontal, 2) ear tip resting on ground, 3) erect, and 4) flat on the ground. The average percentages of rot-damaged kernels were respectively: 1.4, 2.2, 2.9, and 6.9. Rots caused by *Diplodia zeae*, *Fusarium moniliforme*, and *Gibberella zeae* all ranked in the same order. *Nigrospora oryzae* was especially prevalent in lodged ears. In the first three classes, ears well covered by husks had less rot than those with exposed ear tips. In a 5-year test with various single-cross corn hybrids, ears were exposed at the tip ends at 10-day intervals by pulling back the husks by hand. Early husk openings greatly increased rot by the first three fungi mentioned above. After the kernel moistures were down to 35 per cent, the differences between the opened and the check were insignificant. While rot increased in all hybrids by early husk openings, the hybrids still continued to differ greatly in amount of rot. This indicated resistance inherent within the ears themselves. But as none were immune, resistance plus good husk coverage and ear declination downward resulted in least rot damage.

Some factors affecting wilt in a flax-wilt nursery. KOMMEDAHL, T. For a given variety of flax grown in a wilt nursery, there is sometimes a 100 per cent increase in wilt from one season to the next. Although a few of the 40 varieties and selections tested were resistant for 3 years, many were resistant in only one of those years. In seasons when temperatures were high during the seedling stage, seedling wilt predominated; but in other years when temperatures were high in the post-flowering stage, late wilt prevailed with the same varieties. More plants grown from cracked seed wilted than did those from sound seed of the same seed lot. Fewest plants of Punjab wilted on peat, more on silt loam, and most on sandy loam. Punjab grown on wilt-sick soil wilted more when soil was packed than when loose. Less wilt occurred when wilt-sick soil was diluted with ordinary soil, although plants wilted even at 1: 500 dilution. Lime and superphosphate reduced wilt in the wilt nursery but nitrogen, potash, and sulfur were ineffective. High soil moisture favored wilt in Koto, but reduced wilt in Crystal. Since resistance is relative, any one of these factors, or a combination of these and other factors, can significantly influence varietal reaction.

Identification of bolting disease of carrots. KUNKEL, L. O. In 1945 a shipment of diseased carrots was received from Dr. J. D. Menzies of the Washington Agricultural Experiment Station at Prosser, Washington, for identification. One of two packages contained carrots that had shown bronzing and chlorosis of foliage. These roots were affected by aster yellows. The other package contained carrots that had bolted (flowered prematurely and abnormally). This disease was taken to healthy carrot plants by dodder, *Cuscuta campestris*. It could not then be identified, but later was suspected of being caused by the tomato big bud of California and other western States. Tomato big-bud disease received from Dr. L. C. Cochran of the Citrus Experiment Station, Riverside, California, in 1947, was transmitted to healthy carrots. It caused bolting and other symptoms similar to the carrot disease. When the disease from Washington was transmitted to tomato plants, it caused typical big-bud symptoms but was considerably milder than tomato big bud from California. It was concluded that the bolting disease of carrots in Washington was caused by a big-bud virus that was not identical with tomato big bud of California.

Control of oak wilt in certain local areas. KUNTZ, J. E., AND A. J. RIKER. In accord with the discovery of extensive natural root grafting and of passage by the fungus from tree to tree through such grafts, local control of oak wilt in certain Wisconsin areas has been accomplished. Immediate removal of each wilting tree has retarded the disease in 11 out of 18 eradication plots. In four of these plots, no new wilt has appeared for 2 years. Where single, newly wilting trees were poisoned, no spread has occurred either in six 1-year-old plots or in three 2-year-old plots. Poisoning healthy trees bordering wilted areas has prevented further local spread in 12 plots, of which six were four years old. The severing of all root connections both mechanically and chemically has been tried. In ten plots, trenching between wilting and healthy trees has prevented further spread for 2 years. The Wisconsin Conservation Department has developed a tractor-drawn knife that moves 100 ft. per min. and cuts roots to a depth of 36 in. This root-cutter has been used in 12 plots in 1950.

Variation in virulence of potato virus Y isolates from different sources. LARSON, R. H., AND J. F. DARBY. Under greenhouse conditions 18 isolates of the potato virus Y obtained from field material from various sections of the United States and from other laboratories differed markedly in type and severity of symptoms produced as well as in relative virulence. Some isolates were virulent to the extent of killing certain potato varieties while affecting others only slightly. When all isolates were compared on the basis of severity of symptoms produced on 18 American potato varieties, the disease index ranged from 42 to 78. Placid, Cobbler, La Soda, Kennebec, Russet Burbank, Pontiac, Sebago, and Green Mountain were the most tolerant; Russet Rural, White Rose, Essex, and Katahdin were intermediate; and Triumph, Chippewa, Red Warba, White Cloud, USDA seedling 41956, and Ontario were the most susceptible. All 18 isolates in tobacco leaf tissue, when desiccated and stored over calcium chloride at 4° C., remained viable up to 6 months at the last testing. On tobacco, veinclearing accompanied by spot necrosis was produced at 24° C. by all isolates, in combination with a mild mottle strain of potato virus X. No immunity to all virus Y isolates was found in 30 wild and cultivated species of *Solanum* from South and Central America. *Physalis peruviana* L., *Lycium halimifolium* Mill., and *L. rhombifolium* (Moench) Dippel were good local-lesion hosts for most of the isolates.

Blackpatch of red clover is seed-transmitted and causes seedling blight. LEACH, J. G., AND E. S. ELLIOTT. The mycelium of the blackpatch pathogen may infect and kill the seeds, but in many cases infected seeds remain viable. The mycelium usually invades the seeds through the hilum, and in some cases the large mycelium can be seen just under or on the surface of the seed coat. The fungus is readily isolated from such seeds when they are plated on water agar. When infected seeds are germinated in sand in a moist chamber, the mycelium grows out of the seed coat, infects the young seedling, and kills it. The mycelium spreads rapidly from one seedling to another when the seeds are germinated in a moist chamber. The amount of seedling blight was greatly reduced, but not completely controlled, by dusting infected seed with Arasan. Species of *Alternaria* and *Cladosporium* also frequently invade the seed coat of red clover seeds but they are not pathogenic to seedlings.

The inhibition of virus symptom expression by sodium azide, potassium cyanide, and two antibiotics. LEBEN, CURT, AND R. W. FULTON. In these studies detached primary leaves from cowpeas grown under controlled light and temperature conditions were used. Leaves were inoculated on the upper surface with tobacco-necrosis virus or tobacco-ringspot virus, rinsed with water, and placed with the lower side in contact with the surface of an agar medium in shallow plates. The medium contained, in gm. per liter: glucose, 40; KH_2PO_4, 4.5; agar, 10. After 3 to 4 days at 22° to 24° C. in the dark, typical local lesions were produced; they were not produced if glucose was omitted from the medium. The effect of various substances on the development of the lesions was tested by adding them to the medium. Those materials that did not inhibit lesion production by either virus were, in mg. per ml.: antimycin A, 0.02; bacitracin, 1.0; chloromycetin, 1.0; neomycin, 0.5; penicillin G (K salt), 0.37; and streptomycin sulfate, 1.0. Materials that prevented symptom expression by both viruses were NaN_3, 0.65; KCN, 0.65; streptothricin HCl, 1.0; and terramycin HCl, 1.0. Streptothricin and terramycin at 1 mg. per ml. did not inactivate the viruses *in vitro*.

An antibiotic that inhibits certain phytopathogens. LEBEN, CURT, G. J. STESSEL, AND G. W. KEITT. In agar-plate screening tests an unidentified species of *Streptomyces* bearing tight spore spirals was shown to have a marked antagonistic effect on a number of phytopathogenic fungi. An antibiotic material was produced by this organism in a liquid medium (gm. per liter: soybean meal, 40; glucose, 20; $CaCO_3$, 1.5) in shaken flasks

and in small tank fermentors. Activity was assayed by a plate test employing blotting-paper disks and the fungus *Glomerella cingulata*. The active material was obtained in the ethanol extract of the precipitate formed when the pH of the culture filtrate was brought to 3.0. A partly purified product of further fractionation was used in agar-streak tests. All 16 fungi tested, including phytopathogens and *Trichophyton mentagrophytes*, were completely inhibited by 0.47 to 15.0 μg. per ml. Certain bacteria were unaffected by 60 μg. per ml.; others were completely inhibited by smaller amounts. Tests with partly purified preparations indicated that the antibiotic is soluble in methanol, ethanol, and water more alkaline than pH 4.0 to 5.0; slightly soluble in acetone, *n*-butanol, and chloroform; and insoluble in ethyl ether and ethyl acetate. Molisch, Hopkins-Cole, biuret, ninhydrin, Millon, xanthoproteic, and $FeCl_3$ tests were negative.

The effect of ring spot and yellows on the yield of Montmorency cherry. LEWIS, F. H. Orchard surveys since 1945 on 245 sour-cherry trees were checked by indexing all trees on peach in 1949, 108 trees on peach and cherry in 1947, and fewer trees in other experiments. Identification of yellows trees was facilitated by cool weather in the spring of 1950. Classification into groups, with the omission of all trees with any abnormal record other than indicated, gave the following average yields of fruit in pounds per tree in 1950: healthy, 51 trees, 177.3; one-fourth of tree showing shock symptoms ring spot, 6 trees, 166.6; half of tree showing shock symptoms ring spot, 16 trees, 132.2; three-fourths of tree showing shock symptoms ring spot, 25 trees, 111.6; entire tree showing shock symptoms ring spot, 40 trees, 103.8; ring spot present without 1950 symptoms, 7 trees, 172.3; recurrent symptoms ring spot, 18 trees, 159.3; average of 65 per cent of tree showing shock symptoms ring spot in 1950 plus yellows first observed in 1950, 16 trees, 126.1; yellows probably for life of tree, 10 trees, 108.5. The yield reduction by ring spot was greatest the first year symptoms appeared and proportional to the severity of the symptoms. Maximum yield reduction by yellows occurred several years after infection.

Effect of controlled low temperature on the emergence of seedlings from artificially dried seed corn. LIVINGSTON, J. E. The ability of seed corn to produce seedlings following exposure to a soil temperature of 40° F. is greatly reduced in nonsterile soil but only slightly affected in sterile soil. Soil-borne pathogens are primarily responsible for stand reduction. Low soil temperatures, harvesting when the seed has a high moisture content, and artificial drying predispose seedlings to the attack of microorganisms in the soil. The last two factors apparently exert their effect on the seed in such a way that the susceptibility of the young developing seedling is increased. Normal emergence is obtained when the seed is treated with Arasan prior to planting, irrespective of the drying method or moisture content of the seed at time of harvest.

Nematodes found in New York State potato fields with different cropping histories. LOWNSBERY, B. F., J. W. LOWNSBERY, AND W. F. MAI. Comparison was made of the kinds and numbers of nematodes present in several fields where potatoes had been grown continuously for 20 to 30 years, and other areas of the same soil type where they had been grown a short time, or not at all. Nematodes were removed from the soil by screening instead of by use of the Baermann funnel, because it was determined that screening yields approximately ten times the number of plant parasites obtained with the Baermann funnel. A species of *Pratylenchus* was found in each of the fields sampled. *Xiphinema americanum*, *Criconemoides* sp., and a *Pratylenchus* species were found in woods adjacent to a field in which potatoes had been grown continuously for 20 years, but only the *Pratylenchus* species was found in this field. When potato roots grown in soil from the various fields were examined, two species of *Pratylenchus* were the only nematodes found in quantity. Results of this survey indicate that high populations of pathogenic nematodes do not always result from continuous cropping with potatoes.

Solanum xantii and S. integrifolium, new hosts of the golden nematode, Heterodera rostochiensis. MAI, W. F. In testing species of the Solanaceae for susceptibility to the golden nematode, *Heterodera rostochiensis* Wollenweber, seedlings were transplanted to 4-in. pots of soil heavily infested with cysts of this organism. After immature females became evident on the roots of potato, planted at the same time, roots around the perimeter of the soil ball in each pot were examined five times at weekly intervals. When the five readings were negative, all soil was washed from around the roots and the entire root system examined for the presence of immature females. An average of 46 immature females per root was found for *S. xantii* and 13 for *S. integrifolium*. In the same test an average of 713 immature females were present on roots of *Solanum tuberosum* L., 41 on *Lycopersicon esculentum* Mill., and 22 on *Solanum dulcamara* L., previously recognized hosts. At least ten plants of each species were included. *S. xantii* is native to California and *S. integrifolium* is grown in various parts of the United States as an ornamental.

Serial passage of aster-yellows virus through the aster leafhopper. MARAMOROSCH, KARL. Injection of juices from viruliferous to virus-free leafhoppers of the species *Macrosteles divisus* Uhler enabled Black to transmit aster-yellows virus and to obtain evidence for its multiplication in the insect. This technique has now been modified for serial passages through leafhoppers. Viruliferous insects were macerated at 0° C., the pulp diluted 10^{-3} in 0.25 M buffered saline and centrifuged at 3000 r.p.m. for 10 min. By means of a calibrated microsyringe, 1/8000 cc. of the supernatant fluid was injected into individual virus-free leafhoppers. After the insects fed for 30 days on rye, a plant immune from aster yellows, they were caged individually for a 48-hr. test feeding on asters, then macerated, diluted, and used for injecting the next group of virus-free leafhoppers. Control insects proved virus-free. This procedure has been repeated five times and positive infectivity data are available for the sixth transfer. The estimated dilution used to inoculate the sixth group in the series, if no multiplication had occurred, would be at least 10^{-36}, but aster-yellows virus in insect juices has not been infective at dilutions higher than 10^{-3}. The experiment provides further evidence for multiplication of this plant virus in its insect vector.

Orchard experiments on the control of bacterial spot of peaches. MASON, CURTIS L. Tests were on Elberta trees in the Nashville, Arkansas, area: on 8-year-old trees in 1949, and on 4-year-old trees in 1950. In 1949, twelve different treatments were applied when trees were in the full pink stage. Of these treatments, the most effective were sodium hypochlorite at 1 gal. of 5.25 per cent solution per 100 gal. water, and fixed copper compound (50 per cent copper) at 3 lb. per 100 gal. water. Similarly, 16 treatments were studied, involving variations in time of application and materials. Of these tests zinc Bordeaux 6–8–100 (zinc sulfate, 36 per cent zinc), applied weekly from petal fall to one month before harvest, was outstanding. For the 1950 tests, two concentrations of powdered copper sulfate (4 and 8 lb. per 100 gal. water) were applied October 18, 1949. Marked reduction in infection was observed with these treatments. The pink spray results showed some control in all treatments. Of the foliage sprays, zinc Bordeaux (6–8–100), applied at weekly intervals from petal fall until 1 month before harvest, again was outstanding.

Inhibition of growth of Schizophyllum commune by extractives from living trees. MCDONOUGH, E. S., AND LOIS BELL. The fungus *Schizophyllum commune* Fries grows well on dead parts of various species of trees but is not a serious parasite of living trees of these same species. Growth of the fungus was evident after 16 days on sterilized wood and bark taken from 10 species of common, living trees, but the wood and bark of *Catalpa speciosa* Warder and *Pyrus communis* L. supported abundant growth only after prolonged storage of the wood. When two pieces of filter paper impregnated with an alcoholic extract from *C. speciosa* were placed on potato-dextrose agar in Petri dishes and the fungus was inoculated on the agar equidistant from the papers, growth was inhibited according to the concentration of the inhibitor. Inhibition was inversely proportional to the number of days the wood had been cut from the tree. A similar but less active extract has also been obtained from *Ulmus americana* L., a tree which upon death may soon be attacked by *S. commune*. It would thus seem that the wood and bark of some living trees contain a water-labile, alcohol-stabile antibiotic which protects the tree during its life but which rapidly loses its fungistatic powers upon death of the tree.

Soil amendments in relation to the Actinomycete population and the antibiotic index of sugar-cane soil. MCGAHEN, J. W. Alluvial soil from the sugar-cane belt of Louisiana was amended with bagasse compost, cowpea trash, soybean trash, bloodmeal, tankage, bonemeal, and ammonium sulfate. The Actinomycete populations of the amended soils were determined by dilution techniques, and the antibiotic indices were determined from the inhibition of *Pythium arrhenomanes* in culture by the Actinomycete isolates. Significant increases of the populations were obtained from the use of cowpea and soybean trash, bloodmeal, and tankage. The antibiotic index was increased by bagasse compost, cowpea trash, and soybean trash. A decrease of the antibiotic index occurred when the soil was amended with bonemeal, bloodmeal, or tankage. Ammonium sulfate did not materially affect either the Actinomycete population or the antibiotic index. It is considered that the nitrogenous materials, bloodmeal and tankage, caused an increase of the nonantibiotic and/or weakly antibiotic Actinomycetes in favor of the moderately and highly antibiotic Actinomycetes. The cellulosic materials, cowpea trash, soybean trash, and bagasse compost, caused an increase of the antibiotic over the nonantibiotic Actinomycetes.

Factors conditioning sexuality in a Glomerella from Ipomoea. MCGAHEN, J. W., H. E. WHEELER, AND S. J. P. CHILTON. Loci on at least two chromosomes have been found to affect sexuality in *Glomerella*. Genes at two loci, A and B, produce either self-fertile,

partially self-sterile, or heterothallic cultures which may or may not produce perithecial fundaments. Locus A controls the production of sexual structures, and the number and arrangement of these structures are controlled by locus B. Genes at these two loci also control self-fertility in single cultures as well as self-fertility, cross-fertility, and mating reaction in paired cultures. It appears that these two loci are the principal ones which condition sexuality in this organism; however, more than 30 other mutant genes have been studied which affect some phase of the sexual process. Most of these genes are biochemical mutants, which, when present, render normally self-fertile cultures self-sterile. In general, biochemical deficiencies reduce selfing in matings as well as in single cultures. Since the only requirement for crossing is the presence of a suitable gene at locus A, sexuality studies in this fungus can be conducted with crosses between two homothallic cultures, between two heterothallic cultures, and between a homothallic culture and a heterothallic culture. Hence, sexuality in *Glomerella* appears to be conditioned by many factors.

Fusarium wilt of muskmelons and watermelons in southwestern Ontario. MCKEEN, C. D. Within the last 8 years Fusarium wilt has invaded and spread throughout the muskmelon-growing areas of Essex County. In 1948, Fusarium wilt of watermelons was observed for the first time in this area, affecting several plants in two fields in which the muskmelon organism was known to be present. Several monosporous isolates obtained from wilted muskmelon and watermelon plants growing in the same field and in widely separated farms were tested for pathogenicity on both hosts. All isolates were highly virulent on their original host. Moreover, many isolates from muskmelon caused appreciable pre-emergence killing and wilting of watermelon seedlings. Similarly, watermelon isolates have proved capable of attacking muskmelons. The rate of wilting of watermelon seedlings indicated that the three isolates from that host differ pathogenically. Similar tests have failed to reveal any significant differences in pathogenicity among ten muskmelon isolates. The Iroquois variety of muskmelon, which is highly resistant when transplanted into infested soil in the field, was completely susceptible at the seedling stage, in fact even more susceptible than the variety Hearts of Gold (Hoodoo).

A corn root- and stalk-rot complex hitherto known as Gibberella zeae stalk rot. MCKEEN, W. E. In the fall of 1949, root and stalk rot of corn, which has been previously designated as *Gibberella zeae* stalk rot because of the pinkish-red discoloration of the infected tissue, was present in all fields of corn throughout Ontario. This disease has a marked deleterious effect on both the quality and quantity of the grain, and the potential corn yield may be reduced by 50 to 60 per cent. Hybrids show a wide range of resistance and the inbred LC19, used extensively in these studies, proved to be completely susceptible. Infection begins when the ears are in the milk stage and is correlated with ontogeny. First indications of disease are water-soaking of some of the roots and blackening of the lower nodes and frequently also the bundles. This is followed by a brownish discoloration, water-soaking of the first and sometimes the second internode above the crown, and sudden drying of the leaves. A pinkish-red discoloration usually moves up the stem slightly behind the brown water-soaked lesion, and soon the pith is rotted away and only mycelium covers the bundles. A white, rod-shaped, Gram-negative, non-acid-fast, motile bacterium proved to be the first organism present; it was closely followed by *Pythium arrhenomanes* in the brown water-soaked area, and a *Fusarium* sp. in the reddish zone and on the epidermis of the lesion.

A laboratory method for testing reaction of corn to stalk-rotting organisms. MICHAELSON, MERLE E. Surface-sterilized, 3-in. sections of internodes of corn plants 6 to 8 weeks old were inoculated near one end with *Diplodia zeae* and *Gibberella zeae*, and placed across supports in a moist chamber containing a small amount of sterile water. After 1 to 2 weeks at room temperature (20°–25°C.) the sections were cut longitudinally and examined for internal rot. Results in general were correlated with field and greenhouse inoculations on growing plants. On the basis of the amount of internal rot, hybrids and inbred lines of corn differed greatly in their susceptibility to *D. zeae* and *G. zeae* and also to different races of these organisms. This method provides uniformity of light, temperature, and humidity for the development of rots. Numerous tests can be made in a relatively short time from material grown in a small space in the greenhouse, and different parts of a plant can be tested under similar conditions.

The growth-stimulating properties of a metabolic product of Fusarium moniliforme. MITCHELL, J. E., AND C. R. ANGEL. A metabolic product formed during the fermentation of *Fusarium moniliforme* Sheldon causes a marked increase in the rate of development of internodes of several species of plants. The best medium for the fermentation was a simple medium using Cerelose as a source of carbohydrate and ammonium salts as a

source of nitrogen. Plant response was the same whether application was made via root, petiole, leaf blade, or apical bud. Most of the testing has been done with the Valentine bean. Environmental conditions, such as temperature and light intensity, prevailing during the period of the test markedly affected the bean plant's response to treatment. Experiments made under different light intensities have indicated that the active material antagonizes the inhibitory action of light on elongation of bean internodes. Similarly, repression of elongation caused by small doses of indoleacetic acid was counteracted by this metabolite of *F. moniliforme*.

Relation of prune dwarf to sour-cherry yellows. MOORE, J. DUAIN. In both a greenhouse and a field experiment, Italian prune trees were inoculated with buds from Montmorency sour cherry that had 1) sour-cherry yellows with latent necrotic ring spot, 2) sour-cherry yellows with recurrent necrotic ring spot, or 3) latent necrotic ring spot only. In the field experiment, Italian prune trees also were inoculated with buds from Montmorency that had green ring mottle with latent necrotic ring spot. Prune-dwarf symptoms occurred on all Italian prune trees that had been budded from any source of Montmorency that had cherry yellows with latent necrotic ring spot. Prune-dwarf symptoms were not produced on any of the other inoculated trees, nor on any of the noninoculated controls. In the greenhouse experiment, cherry yellows was transmitted back to Montmorency from all Italian prune trees that had been inoculated with buds from any source of Montmorency that had sour-cherry yellows with either latent necrotic ring spot or recurrent necrotic ring spot. Necrotic ring spot only was recovered from those prune trees that had been inoculated with Montmorency buds from trees that had necrotic ring spot only, and neither necrotic ring spot nor yellows was recovered from noninoculated Italian prune.

Cross inoculation of Prunus with Coccomyces. MOWRY, JAMES B. Representatives of 66 species, varieties, and hybrids of *Prunus* were each inoculated in the greenhouse with conidia from single-spore cultures from one or more of the following hosts: *P. avium*, *P. cerasus*, *P. domestica*, *P. fruticosa*, *P. mahaleb*, *P. pensylvanica*, and *P. serotina*. The cross-inoculation groups established by Keitt et al. (Wisconsin Agr. Exp. Sta. Bul. 132. 1937) were, in general, confirmed. *P. fruticosa* should be added to group 1. *P. cerasus*, *P. fruticosa*, and *P. mahaleb* proved susceptible to the *P. serotina* isolate. Similarly, *P. avium* and *P. cerasus* were susceptible to the *P. pensylvanica* isolate. Thus, *P. serotina* and *P. pensylvanica* in nature may serve as potential sources of inoculum for sour cherry. *P. cerasus* seedlings ranged from immune to fully susceptible to the *P. mahaleb* isolate. Seedlings of *P. cerasus*, *P. insititia*, *P. mahaleb*, and *P. tenella* were susceptible to most isolates tested. On the other hand, seedlings of *P. besseyi*, *P. japonica*, *P. pumila*, *P. persica*, *P. salicina*, *P. serotina*, *P. spinosa*, and *P. virginiana* were susceptible to relatively few isolates. Seedlings of *P. glandulosa* and *P. maritima* were resistant to all seven isolates.

Effect of wheat juices on urediospore germination of Puccinia graminis tritici. MUNNECKE, DONALD E. Seedling leaves of Little Club and Khapli wheat were cut, frozen, thawed, and pressed at 8000 lb., and the juices obtained were stored at $-30°$ C. The juice was thawed at room temperature, diluted with water agar, and poured into Syracuse dishes. Fresh urediospores of races 15B, 17, 36, 56, and 167 of *Puccinia graminis tritici* were scattered on the agar surface, and germinated spores were counted after 3 hr. Juice from Little Club, a highly susceptible variety, was much more inhibitory to spore germination than was juice from Khapli, a highly resistant variety. The inhibition was not due to pH or osmotic effects. The inhibitory substance in Little Club juice withstood desiccation under vacuum, was heat labile, and dialyzable, and was adsorbed on cation, but not anion, exchange resins. For standardization, juice was diluted according to its total nitrogen content, percentage total solids, or dry weight of the diffusate after dialysis. Although spore lots were as uniform as possible, germination percentage varied greatly between experiments. The effect of host juices on spore germination varied with the race of rust used. Spore germination on juices prepared from other wheat varieties was not correlated with the susceptibility of the variety.

Control of mint rust with dust fungicides. NELSON, RAY. Row plantings of Scotch spearmint were dusted in 1948, 1949, and 1950 for the control of rust (*Puccinia menthae*). In Michigan, plantings of peppermint adjacent to rusted spearmints remain rust-free. Control plants were moderately rusted in 1948, rust-free in 1949, and killed by the disease in 1950. In the 1948 trials effective protection against rust was provided by four applications of Fermate, Dithane Z 78–sulfur, and cuprous oxide–sulfur dusts. Dithane without sulfur was much less effective. Under severe rust conditions in 1950 Fermate gave protection much superior to that of other materials. A 2 per cent Actidione dust and tri-

basic copper sulfate-zinc were less effective than Fermate but much superior to Dithane Z 78. Since organic and inorganic sulfur fungicides are broken down and leave undesirable residues in the distilled oil, the Actidione and tribasic copper sulfate-zinc dusts are most likely to be useful in mint rust control.

Control of onion mildew with dust fungicides. NELSON, RAY. In 1946–1950 field tests on onion mildew control the disease was destructive only in 1947 and 1950. In 1947 dusts were more effective than sprays, and maximum mildew control and yield increases were obtained with Dithane Z 78–sulfur and cuprous oxide–sulfur. In 1948, with no mildew, Dithane Z 78–sulfur, cuprous oxide–sulfur, and tribasic copper sulfate–sulfur significantly increased yields over DDT alone. In 1949 below-freezing temperatures in May resulted in low yields and no apparent stimulatory effects of the dust formulations. With subnormal seasonal temperatures and with moisture conditions favorable for mildew, the disease caused marked injury in experimental fields in 1950. The Dithane Z 78–sulfur dust controlled mildew better than did other materials and resulted in highly significant increases in yield. A 2 per cent Actidione–sulfur dust was less effective in mildew control, but produced significant yield increases. The cuprous oxide–sulfur dust was apparently injurious under the cool seasonal conditions. The addition of 2 per cent zinc apparently neutralized this toxicity and resulted in yields higher than those in the control plots. In the seasons when mildew was destructive the Dithane Z 78–sulfur dust gave the most effective protection, with consequent maximum yield increases.

New methods of controlling onion smut. NEWHALL, A. G. Suitable replacements have long been sought for the two control methods universally employed by New York muckland onion growers, e.g., the formaldehyde drip, and seed pelleting with Tersan. For two seasons a Root hand blower mounted on the handles of the drill and operated by chain drive from a front wheel has been successfully used to blow fungicidal dusts into the open seed furrow as it is being closed by the packer wheel. In tests on 30 farms the following gave good control: Tersan, 10 lb. per acre; Tersan plus a diluent (1: 4), 20 lb. per acre; tetramethyl thiuram disulfide (TMTD) plus pyrophyllite (1: 2) and Orthocide, 15 and 20 lb. per acre. Seed pelleted with Orthocide at 1 lb. to 1 lb. of seed also was effective. Most promising was a 1: 4 mixture of Tersan, plus an insecticide, at 22 lb. per acre. This also gave excellent control of the onion maggot. Excellent smut control was obtained also by mixing TMTD with 5–10–10 fertilizer at 60 lb. per ton, broadcast at ½ ton per acre and harrowed lightly into the top inch of muck. Disking this reduced the control.

An economical flash-type continuous soil pasteurizer. NEWHALL, A. G., AND W. T. SCHROEDER. A new, mobile heater and dryer designed for heating sand and gravel has been successfully adapted for soil pasteurization. It is capable of pasteurizing 1 to 2 bu. of soil per min. or 100 an hour, and consists of a revolving, sloping cylinder 8 ft. long and 17 in. in diameter. Soil shoveled into the upper end is tumbled against the hot sides of the cylinder and emerges in a few seconds from the lower end at almost any desired temperature. Heat is supplied by one or two blow torches burning 3 or 4 gal. of fuel oil an hour, mounted so that the flame is directed into the lower end of the cylinder. Final soil temperatures are governed by original soil temperature, soil moisture, slope of the cylinder, speed of shovel operator, and size of flame, the last three being easily altered to suit local conditions. Soil productivity and organic matter were not impaired. Excellent control of damping-off and weed seeds has been obtained at a fuel cost of less than 1 cent per bu. of soil. Seeds may be sown the same day soil is heated. Tests on the control of Verticillium in compost soil have been conducted at the Geneva Station.

Influence of temperature on the development of internal-cork symptoms in sweet-potato roots. NIELSEN, L. W. Porto Rico sweet-potato roots infected with internal cork were collected in October, 1949, and cured 12 days at 85° F. Before and after curing, approximately 60 per cent of the roots had the cork symptom. On November 29 one lot of roots was stored at 55°–60° F. and another at 78°–80° F. At approximately 6-week intervals until April 20, 1950, 100-root samples were sliced to ascertain the development of the cork symptom. At the higher temperature symptoms developed in 91 per cent of the roots, and the lesions were larger and more numerous than at time of harvest. No symptom changes were detected in roots stored at the lower temperatures.

The relation of Periconia to milo root rot in California. OSWALD, JOHN W. In view of Leukel's report that *Periconia circinata* rather than *Pythium arrhenomanes* causes milo root rot, further studies of this disease were conducted. When susceptible DD (Double Dwarf) and resistant DD38 milo varieties were inoculated with Leukel's isolate of *Periconia circinata*, the former was killed whereas the latter remained healthy. Pure cul-

ture inoculations with *Pythium arrhenomanes* caused reddening of the roots and stunting of equal severity on both varieties. Although isolation of *Periconia circinata* from susceptible DD plants growing in naturally infested California soil was successful, at first all isolates obtained were nonpathogenic even though culturally and morphologically indistinguishable from Leukel's culture. Because conidia could not be induced to germinate, single conidiophores were isolated from rotted roots that had been held in moist chambers. All conidiophores, thus isolated, grew from their basal ends and produced identical-appearing colonies. Approximately half of these single conidiophore cultures were strongly pathogenic, whereas the others were nonpathogenic. All cultural mutants arising from pathogenic isolates—some completely white—retained the pathogenicity of the parental isolate.

Field control of potato scab in California. OSWALD, JOHN W., AND DAVID W. WRIGHT. Potato scab (*Streptomyces scabies*) is very destructive in the California spring crop, especially in Kern County, where a large acreage is out of production because of severe infestation. The most promising means of control is a heavy application of sulphur to the soil. Application of 2500 lb. of sulphur per acre in February 1948 gave the following percentages of marketable tubers: in 1948, 29 (pH 4.8); in 1949, 91 (pH 3.7); in 1950, 92 (pH 4.3). Untreated plots in the same 3 years yielded 23, 12, and 26 per cent of marketable tubers. Total yields were not significantly affected in 1948 or 1949. Untreated plots yielded slightly higher in 1950. Applications of 1000, 1500, 2000, and 2500 lb. of sulphur per acre in September 1948 all failed to control scab the first year. In the second crop, however, the percentages of marketable tubers for the four sulphur rates were 67 (pH 5.2), 72 (pH 4.7), 83 (pH 4.5), and 98 (pH 4.2), respectively. Addition of 2 tons of lime to sulphur-treated soil (pH 4.0) raised the pH to 6.5 without any increase of scab in the subsequent crop. In this area cotton fulfills the need for a crop that will grow successfully the first year after sulphur treatment of the soil.

The pathological effects of Belonolaimus gracilis on peanuts in Virginia. OWENS, JOHN V. The sting nematode, *Belonolaimus gracilis*, has hitherto been reported only from corn and pine roots in Florida. Investigations conducted during the 1950 growing season in the vicinity of Holland, Virginia, indicate that it can cause serious damage to peanut crops. When the infestation is heavy, the lateral roots are gnarled and stubby; frequently only the taproot is left. Such plants show a marked decrease in yield of nuts and peanut hay. Peanuts growing in flasks of agar were inoculated with specimens of *Belonolaimus;* minute necrotic lesions were observed along the taproot as a result of their feeding, and some specimens were observed in the act of feeding on the roots. This nematode also was found attacking cotton, corn, and soybean crops in this area.

Recent changes in the relative prevalence of physiologic races of crown rust in Canada. PETURSON, B. Surveys have been made yearly since 1929 to determine the prevalence and geographic distribution of the physiologic races of *Puccinia coronata* that occur in Canada. From 1929 to 1945 races 1, 2, 3, 4, 5, and 6 were the predominant ones. Each year during that period these six races comprised 95 per cent or more of all isolates identified. In 1938, the first isolates of the races that are capable of attacking varieties possessing the Bond type of crown-rust resistance were identified. These races (34, 45, and 57) were isolated only occasionally from 1938 to 1945 and during that period comprised a negligible portion of the physiologic races of crown rust present in Canada. In 1946 and 1947 there was a noticeable increase in the prevalence of these races, and each year since that time there has been a substantial increase. In the years 1948, 1949, and 1950 they comprised 18, 47, and 70 per cent, respectively, of all isolates identified. This increase in the prevalence of these races in Canada coincides with an increase in acreage, in the United States, of new oat varieties possessing the Bond type of crown-rust resistance.

Comparative pathogenicity of two Colletotrichums on Easter-lily bulbs. PLAKIDAS, A. G. In 1944 the writer described *Colletotrichum lilii* sp. nov. as the cause of "black-scale" disease of Easter-lily bulbs. A second Colletotrichum is associated with a dieback of the tips of lily bulbs in Oregon, and several inquiries have been received from lily growers, pathologists, and inspectors as to whether or not this is the same as *C. lilii*. Healthy bulbs of *Lilium longiflorum* were planted in parallel series of sterilized and non-sterilized soil infested with pure cultures of *C. lilii* and two separate isolates of the Oregon Colletotrichum, one isolated from Oregon-grown bulbs by A. Schlocker, California Department of Agriculture, and the second by the writer from a Croft lily bulb sent by Dr. F. P. McWhorter, Corvallis, Oregon. The two Oregon isolates appeared alike in culture but were used separately. *C. lilii* caused severe black-scale symptoms on every bulb grown in both sterilized and nonsterilized soil. In contrast, neither of the two Oregon isolates

caused black-scale symptoms. Culturally the Oregon Colletotrichum differs from *C. lilii* in rate of growth and color of mycelium, but the conidia of both are similar in size and shape. It is concluded that the Oregon lily fungus is different from *C. lilii*.

A Fusarium wilt of radish in Wisconsin. POUND, GLENN S., AND DORSEY L. FOWLER. A Fusarium wilt of radish has recently become very severe in muck soils of southeastern Wisconsin. Initial symptoms consist of yellowing and unilateral curvature of leaves, generally followed by death of the plant. Suppression of root enlargement is very marked and vascular tissue of infected roots is conspicuously darkened. In parallel inoculations with *Fusarium oxysporum conglutinans*, the incitant of cabbage "yellows," at a soil temperature of 24° C., both isolates were infectious to radish, mustard, Chinese cabbage, turnip, rutabaga, kohlrabi, stock, garden cress, and wallflower. In addition to these hosts, the cabbage-yellows organism attacked cabbage and other members of the cabbage tribe which the radish organism did not infect. However, at soil temperatures of 28° and 32° C., the radish organism infected a high percentage of cabbage plants of varieties susceptible and resistant to *F. oxysporum conglutinans*. Disease development in cabbage was mild and stunting was the main symptom. All American varieties of radish were susceptible to the radish organism, the winter varieties being slightly more tolerant than the others. Resistance in the Scarlet Globe variety has been developed by selection. Preliminary experiments indicate that the radish organism is identical with *F. oxysporum raphani* described from California by Kendrick and Snyder.

Production of a toxic substance by Alternaria solani and its relation to the early-blight disease of tomato. POUND, GLENN S., AND M. A. STAHMANN. A metabolic substance of *Alternaria solani* produces chlorosis and necrosis when introduced into the tomato plant from fungus lesions on the plant or as culture filtrates. Unilateral chlorosis and necrosis of leaflets distal to petiole lesions are common. Lesions on the main stem, either at the soil level or in the central portion, may produce necrosis or chlorosis of terminal leaves. Indistinguishable symptoms were produced by introducing sterile filtrates into lower petioles of growing plants. When cuttings were placed in sterile filtrates rapid epinasty, wilting, and necrosis developed. The toxic substance was more active when taken in by roots than when sprayed on foliage. Highly active concentrates of the toxic material had been prepared when Brian et al. in England reported on the isolation of Alternaric acid from *Alternaria solani*. A preparation of crystalline Alternaric acid showed typical phytotoxic activity at concentrations as low as 0.1 microgram per ml. Symptoms produced by the toxic substance are somewhat similar to field symptoms of the disease, and it is suggested that the symptom syndrome of the disease may be due in part to the material *per se*.

Vapor-phase toxicants for Botrytis sp. RICH, SAUL. Materials were tested as vapor-phase toxicants against both the spores and mycelium of *Botrytis* sp. Mycelial growth and spore germination were effectively inhibited by the presence of p-dichlorobenzene, m-nitrochlorobenzene, p-nitrochlorobenzene, pentachloronitrobenzene, tetrachloronitrobenzene, and crotonaldehyde. Removing the toxicants allowed mycelial growth to resume in all the dishes that had contained tetrachloronitrobenzene and pentachloronitrobenzene, in some of the dishes that had contained p-dichlorobenzene and m-nitrochlorobenzene, but in none of the dishes that had contained crotonaldehyde. Some of these materials may be of value as fumigants for stored nursery stock that is susceptible to damage by *Botrytis* sp. Physiological differences between mycelium and spores are indicated by their response to both tetrachloronitrobenzene and pentachloronitrobenzene. In both cases, a 24-hr. exposure was completely fungicidal to *Botrytis* spores, while an 8-day exposure was merely fungistatic to the *Botrytis* mycelium. Some materials that proved ineffective in the tests against *Botrytis* mycelium were potassium sulfide, nabam, zineb, and a mixture of chlorinated melamines (50 per cent available Cl_2). The potassium sulfide test demonstrates the tolerance of *Botrytis* mycelium for H_2S, and the chlorinated melamine test does the same for free chlorine. It may be possible to use fumigant fungicides for the control of all storage rots.

The spread of oak wilt in local areas. RIKER, A. J. The spread of oak wilt in local areas has been followed in 41 mapped plots established from 1939 to 1949 on various sites in central and southern Wisconsin. Periodic examinations of these plots were made. The progressive, tree-to-tree spread of the disease outward from an original infection center was roughly similar for all plots. Usually a single tree and, in many cases, a dominant tree was initially infected. Radial spread outward usually involved one or two trees per season. Only rarely did black or red oaks escape the outward spread of infection, and these trees usually became infected within a few years. Bur and white oaks growing among red, black, or jack oaks often escaped infection and usually were the only oaks remaining in a wilt area. The disease stopped naturally in only three plots. Natural barriers, such as roads, sometimes have halted local spread.

Chenopodium hybridum, a local-lesion host for cucumber-mosaic virus. ROBERTS, DANIEL A., R. E. WILKINSON, AND A. FRANK ROSS. All isolates of cucumber-mosaic virus thus far tested produced necrotic local lesions on leaves of *Chenopodium hybridum* L. in 7 to 10 days following mechanical inoculation. Carborundum was used in all cases. Twenty-two isolates of the virus were tested, including six distinct strains confirmed by cross protection. Lesions appear as small, water-soaked spots which become necrotic and enlarge to diameters of about 4 mm. Virus has been reisolated from single lesions, but attempts to reisolate it from noninoculated leaves on the same plants have been unsuccessful. Results of preliminary dilution studies where the half-leaf technique was employed indicate that *C. hybridum* can be used in quantitative tests with cucumber-mosaic virus.

A race of crown rust of oats apparently new to the United States. ROSEN, H. R., AND H. C. MURPHY. In the fall of 1949 a race of *Puccinia coronata* was found in Arkansas that appears to be somewhat similar to race 55 previously found in Argentina. Bond has a susceptible reaction to it, and Victoria has an intermediate to susceptible reaction in the greenhouse, but only an intermediate reaction out of doors. Most Victoria derivatives have reactions similar to Victoria with a few possible exceptions, notably Fulgrain 8 which appears fully susceptible. Under natural conditions, Landhafer, Ukraine, Rainbow, Santa Fe × Clinton C. I. 5400, Shands' C. I. 4508, and Rosen's selections of R22–36b–9 are resistant. Under similar conditions the following, in addition to Bond, are susceptible: Richland, Minrus, Mindo, Canuck, Clinton, Cherokee, Nemaha, Colo, Bonda, Columbia, Benton, Colo × Fultex C. I. 5395, Victorgrain '50 breeder's stock, Markton, Bonham, Shelby, Andrew, Forward × (V–R) × Forward, Ithacan × Victoria, Columbia × (V–R) C. I. 5297, Columbia × Marion, LeConte, and (Iowa D69–Bond) × Fultex.

Production of ethylene by virus-infected plants. ROSS, A. FRANK. Leaves of several plant species infected with certain viruses produced a physiologically active emanation in amounts much greater than that produced by comparable healthy leaves. The emanation, presumably ethylene, was detected and measured biologically by the triple response of etiolated pea seedlings. Inoculated leaves were used in all tests. The virus-suscept combinations in which abnormal ethylene production was detected are potato virus Y in *Physalis floridana* and U.S.D.A. potato seedling 41956; potato virus X in *Gomphrena globosa;* tobacco-mosaic virus in *Nicotiana glutinosa* and *Datura stramonium;* alfalfa-mosaic virus in *Phaseolus vulgaris;* and tobacco ring-spot virus in *N. tabacum.* Ethylene production appeared to be associated with necrosis. It was greatest in leaves inoculated with viruses that cause numerous large necrotic lesions. In plants of *Physalis floridana* infected with virus Y, ethylene production was greater at 70° F., a temperature favorable to the development of necrosis, than at 80° F., a temperature favorable to virus multiplication without development of necrosis. Epinasty was noted in those cases in which large amounts of ethylene were detected; that is, in the species that normally respond by epinasty to exposure to ethylene.

Population dynamics of nematode parasites of tobacco in certain crop rotations. SASSER, J. N. Population studies of root-knot (*Meloidogyne incognita*) and meadow nematode (*Pratylenchus* sp.) were made on 2-year rotation plots of cotton-tobacco, corn-tobacco, peanuts-tobacco, weeds-tobacco, oats-weeds-tobacco, and tobacco-tobacco. Soil samples from the individual plots were taken to the greenhouse, where degree of infestation of both root-knot and meadow nematodes was measured by growing indicator crops of tobacco and corn. Sampling dates were February, May, and November of 1949, and March 1950. Both root-knot and meadow nematode populations fluctuated widely. Root-knot populations increased to about the same extent in both tobacco and corn plots. Where peanuts followed tobacco, a high root-knot nematode population was reduced to a trace. Where tobacco followed peanuts, the population built up again. In cotton-tobacco rotation plots, root-knot nematodes were more abundant after cotton than after tobacco. Moderate populations of meadow nematodes were present after tobacco in all rotations, whereas lower populations were found after crops of peanuts, weeds, and oats-weeds. Meadow nematodes were very abundant after both corn and cotton, indicating the suitability of these crops as host plants for *Pratylenchus* sp.

Control of golden nematode of potatoes with an organic phosphate insecticide. SASSER, J. N., J. FELDMESSER, AND G. FASSULIOTIS. Systox spray (E-1059), an organic phosphate insecticide, when placed in direct contact with golden nematode (*Heterodera rostochiensis*) cysts in Petri dishes for 1 day or for 1 week, inhibited larval hatching. After 1-day treatment, inhibitions ranged from 10 days for cysts treated with 0.25 per cent up to 61 days for cysts treated with a 2 per cent concentration. Cysts treated for 1 week had longer periods of inhibition and fewer larvae hatched than in material exposed for 1 day. E-1059 at concentrations of 0.25, 0.5, 1, and 2 per cent, in direct contact with

larvae for 1 day, killed 6, 6, 36, and 100 per cent, respectively. The percentage killed by a 1-week exposure to 0.25 and 0.5 per cent concentrations was 94 and 99, respectively; 100 per cent kill resulted at concentrations of 1 and 2 per cent. Spraying potato plants growing in infested soil with a 1 per cent concentration did not reduce the number of new cysts.

Variation in pathogenicity of Venturia inaequalis to scab-resistant Malus. SHAY, J. R., AND L. F. HOUGH. Each of eleven scab-resistant clones of Malus was inoculated with eight or more of 34 single-spore cultures of *Venturia inaequalis* (Cke.) Winter. The isolates differed in ability to incite the symptom typical of a given resistant clone. Twenty-three isolates incited pin-point pits on *M. atrosanguinea* (804), but three failed to incite visible symptoms. Nineteen isolates incited pin-point pits on *M. micromalus* (245–38); three failed to incite visible symptoms. Twenty-one isolates incited necrotic nonsporulating lesions on (*M. floribunda* (821) × Rome Beauty) sib; five failed to incite symptoms. One isolate which failed to incite the severe necrosis symptom typical of *M. sikkimensis* transmitted this character on a single-factor basis. All isolates tested, including six from Europe, incited more or less severe symptoms on Jonathan and Red Rome, whereas none of the isolates was able to sporulate on any of the resistant clones. It is hoped that differential isolates may be obtained which will aid in identifying the types of resistance possessed by the various Malus clones. Such isolates will be useful in formulating hypotheses of gene relationships in the scab-resistance breeding program.

Some characteristics of a virus inhibitor in cucumber. SILL, WEBSTER H., JR. When sap from cucumber plants infected with cucumber virus 1 was inoculated to Black cowpea, few local lesions were formed. When the same virus from Havana tobacco was assayed, many lesions resulted. When the yellow-green tissue in infected cucumber leaves, isolated with a small cork borer, was used, high lesion counts were obtained. Sap from green tissue from the same leaves produced few lesions. To check the presence of an inhibitor a standard 1:10 dilution of the virus from tobacco was compared to the same standard plus 1:10 dilution of healthy cucumber sap. The inhibitor was present in all cucumber varieties tested. Various tissues of the plant—leaf, stem, root, corolla, etiolated seedlings, and ungerminated seeds—contained the inhibitor. Maximum inhibition was achieved instantly upon maceration and activity was evident *in vitro* 20 days after extraction. Upon dilution the inhibitor lost potency between 10^{-2} and 10^{-3}. After centrifugation at 70,000 r.p.m. for 1 hr., maximum potency remained in the supernatant liquid. Among other cucurbits tested, watermelon was the only one having a comparable inhibitor.

Some effects of tetramethylthiuramdisulfide on metabolism of conidia of Fusarium. SISLER, H. D., AND C. E. COX. The effect of tetramethylthiuramdisulfide (TMTD) upon respiration and fermentation of conidia of *Fusarium roseum* was investigated by means of the Warburg respirometer. The conidia were washed free of the medium on which they were produced, suspended in modified Czapek's solution, and treated with various concentrations of TMTD. Within the range of 10^{-2}M to 10^{-4}M, TMTD apparently did not affect endogenous respiration. When conidia were treated with 10^{-2}M or 10^{-3}M TMTD before sugar was supplied, exogenous respiration did not become established. The depressing effect upon already established exogenous respiration was proportional to concentration of the TMTD. Reduction of methylene blue by conidia under anaerobic conditions was likewise inhibited in proportion to the concentration of TMTD. Under an atmosphere of nitrogen, TMTD suppressed fermentation. A volatile material produced when TMTD is in contact with conidia of this fungus was capable of inhibiting germination of other conidia not in contact with the TMTD but enclosed in the same vessel. Conidia of *Fusarium oxysporum* f. *batatas* responded similarly to those of *F. roseum* in most respects.

Evaluation of apple fungicides for Iowa. SMITH, F. O., AND W. F. BUCHHOLTZ. Phygon (2,3-dichloro-1,4-naphthoquinone), ½ lb., Puratized Agricultural spray (phenylmercuritriethanol ammonium lactate), 1 pt., Tag (phenyl mercuric acetate), ½ pt., and lime sulfur, 6 lb. per 100 gal., were applied to Jonathan, Starking, and Secor apple trees through the first cover spray. Five cover sprays with Sulforon X followed these treatments. Sulforon X, 6, 4, or 2 lb., Fermate (ferric dimethyl dithiocarbamate), 2 lb., and 341-SC (2-heptadecyl glyoxalidine acetate), 3 pt., were applied throughout the spray schedule. All materials held early scab below 1 per cent; however, infection on Puratized- and Tag-treated trees was less than half that on any others. On September 14 there were less than 5 per cent infected leaves in the Tag, Puratized, and Fermate plots. Tag-sprayed plots contained the least scab throughout the season. 341-SC gave less control than Sulforon X; Phygon and lime sulfur gave less than either. Fruit russeting

was negligible in all plots. Before application of any miticide, European red mite count was lower on 341-SC plots than on any others. On unsprayed trees there were 5.4 and 89.2 per cent of leaves with one or more scab lesions on June 16 and September 14, respectively; early scab development was light at Ames in 1950.

Increase in prevalence of Puccinia graminis tritici race 15B and P. graminis avenae race 7 in 1950. STAKMAN, E. C., AND W. Q. LOEGERING. Race 15B, the most virulent race of wheat stem rust ever found in North America and previously found almost exclusively near barberries, spread and multiplied spectacularly in 1950. It was found in 12 states from Texas to northernmost United States and comprised nearly one-fourth of all isolates. It attacks all hitherto stem-rust-resistant commercial varieties of durum and bread wheat and caused heavy damage to durums in northern Minnesota and North Dakota. Race 7 of oat stem rust, also previously restricted principally to barberry areas, attacks Mindo, Bonda, Clinton, and other varieties of oats deriving their stem-rust resistance from White Tartar. It was found from Florida to Texas and northward to the Canadian boundary in 1950 and comprised almost one-half of all isolates in the United States. Race 8, which attacks varieties with the Richland type of stem-rust resistance, also was widespread and comprised about one-fifth of the isolates. Race 2, which attacks neither of the above groups of varieties, was widespread and comprised the remainder of the isolates. It is possible that these two dangerous races, 15B of wheat stem rust and race 7 of oat stem rust, can now maintain themselves independently of barberries.

Increment curves based on counts of virus particles in plant juices prepared by a new and simplified procedure for electron microscopy. STEERE, RUSSELL L. Curves showing the increment in number of virus particles following inoculation have been obtained for tobacco-mosaic virus (*Marmor tabaci* Holmes) multiplying in plants of *Nicotiana tabacum* L. var. White Burley and for tomato bushy-stunt virus (*Marmor dodecahedron* Holmes) multiplying in plants of *Datura meteloides* DC. Representative samples of infected plant tissue were homogenized, diluted with distilled water, clarified, diluted further with a suspension of indicator particles, and deposited in the form of minute droplets on prepared specimen films. Electron micrographs were taken of numerous drop residues and the numbers of virus and indicator particles in each were counted. From these counts and from the known indicator particle concentration and dilution factors, the concentration of virus particles in each sample was determined. The normal particle increment curves for both viruses resemble standard logarithmic growth curves and curves of autocatalytic reactions. There is an initial stationary phase followed by a logarithmic phase at 20–72 hr., then a final maximum stationary phase. Experimentally altered curves were produced by placing infected plants in a chamber kept at 38° C. These curves show, as anticipated, a pronounced effect of the high temperature upon the multiplication of both viruses.

Methods of selecting soil microorganisms that produce antibiotics active against plant pathogens. STESSEL, G. J. Soil samples were added to a yeast extract-glucose medium at dilutions giving 30–50 colonies per plate and incubated 4 days at 26° C. Dilution plates yielding approximately 70,000 discrete colonies were sprayed with standardized, mixed suspensions of spores of four mutually nonantagonistic phytopathogenic fungi. After 2 days' incubation, 170 culturally different organisms producing clear inhibition zones against all the test fungi were isolated. Streak plate cultures of these organisms were sprayed separately with suspensions made from each of eight phytopathogenic fungi and two phytopathogenic bacteria. Twenty-one of the isolates, mostly actinomycetes, were selected on the basis of comparative inhibition for further work. These organisms were grown in five liquid media in shaken flasks and the culture filtrates assayed for activity against *Glomerella cingulata*, using a modified cylinder plate method. The assay of crude filtrates was facilitated by sealing the cylinders in plates with the upper seeded layer of medium. Sixteen of these organisms produced antibiotic materials in one or more liquid media. The active culture filtrates were tested for pH, time, and heat stability. Of the 170 organisms originally isolated, five appeared to give desirable results in all these screening tests.

Chemotherapeutic control of Fusarium wilt of carnations. STODDARD, E. M. Fusarium wilt of carnations may cause extensive losses in the greenhouse, in spite of the customary precautions. Chemotherapy has been effective in reducing these losses in commercial greenhouses. Materials were applied in water solution to the soil in a bench of Miller's Yellow variety at the rate of 1 pt. per ½ cu. ft. at biweekly intervals for 8 weeks. Each plot comprised approximately 100 plants. In counts made 3½ months after the last treatment, the following percentages of plants were diseased for the several treatments: 8-quinolinol sulfate (1: 4000), 21; 2-norcamphane methanol (1: 16,000), 23;

4-chloro-3,5-dimethyl phenoxyethanol (1: 64,000), 23; copper-8-quinolinolate (1: 4000), 45; check, 44. Three of the materials gave significant reduction of diseased plants. In a subsequent experiment the three successful materials were applied at the same concentration and rate to rooted cuttings from cutting beds to final benching. 2-Norcamphane methanol, 4-chloro-3,5-dimethyl phenoxyethanol, and 8-quinolinol sulfate showed percentage losses due to wilt of 2.85, 3.2, and 4, respectively. The loss in the checks was 50.6 per cent.

A chemotherapeutic control of strawberry red stele. STODDARD, E. M. The red stele disease of strawberry, caused by *Phytophthora fragariae*, has been controlled by soil applications of disodium ethylene bisdithiocarbamate (Dithane D-14). There is evidence that the chemical acted therapeutically and not as a soil sterilant, and thus is a new approach to the control of root diseases. Units of 10 strawberry plants, growing in sand, received five successive applications of each of two concentrations of Dithane D-14, 1.5 and 0.75 per cent, respectively. The plants were then planted in infested untreated soil without further treatment. The higher concentration gave 100 per cent, and the lower 60 per cent, control. The 1.5 and 0.75 per cent solutions, applied in like manner to plants set directly in infested soil, gave 70 and 100 per cent control, respectively. The poorer control of the higher concentration was ascribed to root injury preventing sufficient uptake of chemical for protection. Only 10 per cent of the check plants grown in untreated soil remained healthy. The fact that control was achieved by treating plants grown in sand and subsequently planted in infested untreated soil with no further treatment indicates that the Dithane D-14 was absorbed by these plants and acted therapeutically. In a field trial two subsurface applications of the 1.5 per cent solution completely prevented the spread of the disease from an infested area, at the time of application as well as the following year.

Physiological specialization and mutations in Thielaviopsis basicola. STOVER, R. H. *Thielaviopsis basicola* exists in nature in two distinct forms, termed the brown and the gray wild types. The brown wild type consists of at least two physiological races on tobacco. The mutation from the gray wild type to the brown (race II) occurs only with host passage (tobacco and legume hosts), and the mutant possesses the increased virulence associated with the brown wild type. The reverse mutation from brown to gray occurs in culture and with host passage. Evidence is presented that the mutation from the gray to the brown wild type is associated with the parasitic mode of life of *T. basicola;* also, that it is an essential phase in the evolution for virulence on specific hosts.

Some methods and problems in the study of nematode root rot of tobacco in Ontario. STOVER, R. H. The burley varieties Green Briar and Harrow Velvet are used in Ontario to determine the importance of nematodes in the etiology of the brown root-rot complex of tobacco. The former variety is highly resistant to nematode root rot in naturally infested brown root-rot soil and in nematode-infested Vermiculite, whereas the latter is highly susceptible under similar conditions. Varietal differences in nematode populations per gm. of root are not significant. Both varieties respond similarly to a root rot induced by the addition of decomposing crop residues to the soil, and alleviated by applications of nitrate nitrogen. It has not been found possible to induce consistently or with regularity the symptomatological picture of nematode root rot either in soils where preceding tobacco was heavily infested and where the disease commonly occurs, or in soils that have been cropped with corn and timothy. Of the various procedures used in an attempt to induce nematode root rot regularly under controlled conditions, the timothy-Vermiculite method has proved the most successful. Evidence is presented that typical brown root rot, in which nematodes are a primary etiological agent, can be induced only when certain as yet undetermined soil-host-environmental conditions are present. The timothy-Vermiculite method of building up a large population of root rot-inducing nematodes offers one approach to the problem.

Guatemalan corn as a source of resistance to Helminthosporium turcicum in maize. TAYLOR, G. S., G. SEMENIUK, AND I. E. MELHUS. In Guatemala, certain native corns had high resistance to the indigenous strains of *Helminthosporium turcicum* Pass. Limited tests indicated that this *H. turcicum* was of the same strain as that prevalent in the United States. A program was therefore initiated to incorporate the high resistance of the Guatemalan corns into United States corn. Several United States inbreds were crossed with resistant open-pollinated Guatemalan corn which produced 20 to 26 leaves and did not flower in Ames, Iowa, until early September. The Guatemalan corn, the inbreds, and the hybrids between them were evaluated by artificial inoculations for their resistance to United States cultures of *H. turcicum* in the field and greenhouse during 1948 and 1949 at Ames. In the field the Guatemalan corns proved highly resistant, the inbreds highly susceptible, and the hybrids resistant. Similar responses

were obtained on seedlings in the greenhouse. Advanced hybrid generations segregated for resistance; some of the first backcrosses to the susceptible United States inbred parents were resistant and others susceptible. This reflected the heterozygous condition of the open-pollinated Guatemalan corn used in making the initial hybrids.

Effect of ultraviolet light on the germination of spores of Monilinia fructicola, Cladosporium cucumerinum, and C. epiphylum. THANOS, ANDREW. A study of the effects of ultraviolet light on the germination of four isolates of pathogenic fungus spores indicates the possible practical use of ultraviolet to kill these spores. Spore suspensions, in nutrient broth (Difco), of *Cladosporium cucumerinum, C. epiphylum,* and two isolates of *Monilinia fructicola,* were exposed to the radiations of ultraviolet light, the source of which was a unit consisting of two Westinghouse (G36T6) Slimline Germicidal Sterilamps placed 3 in. above the spore suspensions. At this distance the intensity of light on the suspension surface was approximately 2166 microwatts per square cm. About 95 per cent of the ultraviolet radiations from these Sterilamps are in the 2537 Å unit region. The results, as compared to the controls, show marked inhibition of germination at 15 sec. exposure for the *Cladosporium* spp. and what appears to be complete kill at 2 min.; with the *Monilinia* a significant reduction in the germination, as compared to the controls, was noted at 5 min., with complete kill evident under longer exposures. Further study is necessary to determine the fungicidal efficiency and possible practical application of this agent.

Anthracnose of digitalis. THOMAS, C. A. A serious disease of *Digitalis lanata* has been observed in Pennsylvania in recent years. In 1948, one commercial planting of this drug crop was completely destroyed. The causal organism, identified as *Colletotrichum fuscum* Laub., was found to be seed-borne, to cause damping-off of seedlings, and to be easily spread in the seedbed. Numerous lots of seed from Pennsylvania and Wisconsin were infested. Observations of isolated plantings in Pennsylvania and Maryland indicated the disease could be effectively controlled by starting with clean seed. Hot water (55° C., 15 min.) gave complete control of the organism on the seed and was more satisfactory than several seed-treatment chemicals. The disease developed most rapidly in cool, wet periods. The optimum temperature for growth of the organism in culture was 25° C. The organism was decidedly less tolerant of higher (27–30° C.) than of lower temperatures (15–20° C.). All species of *Digitalis* tested were susceptible. In field plots *D. purpurea,* the commercial source of digitalis in the United States, *D. ambigua,* and *D. sibirica* were considerably less susceptible than *D. lanata* or *D. ferruginea.*

Viruslike particles in strawberry plants with foliar variegation. THORNBERRY, H. H., A. E. VATTER, AND D. M. BEESON. Viruslike particles have been demonstrated by electron microscopy in the juice of strawberry plants having foliar variegation. The particles demonstrable in the juice of plants of a clone not showing variegation for the past 4 years were sparse and irregular in form and size. The viruslike particles from variegated plants are spherical and approximately 35 mμ in diameter, as determined from palladium-shadowed electron micrographs at 20,000× and enlargements to 100,000× for measurements. The particles were fractionated from other smaller constituents of the juice by ultracentrifugation at pH 8.5 in 0.1 M. phosphate buffer. They were fractionated from larger particles, including bacteria, by filtration through Mandler filters at pH 8.5 in 0.1 M. phosphate buffer. Most of the viruslike particles sedimented at 10,000 times gravity for 30 min., less at 15,000, and a few at 20,000. Infectiousness of the particles is being tested.

Delayed sporulation of Colletotrichum on soybeans. TIFFANY, LOIS H. Of the three anthracnose fungi which commonly occur on soybeans, *Colletotrichum truncatum* (Schw.) Andrus & Moore, the form originally discussed as the causal agent of soybean anthracnose, is the most active parasite. When plants of the variety Lincoln were spray-inoculated in the field with a suspension of conidia of this species, however, the fungus was recovered from leaves that had no symptoms of disease. Seed-borne inoculum of *C. truncatum* was responsible for three types of infection: pre-emergence killing, seedling blight, and symptomless establishment of internal mycelium. The third type was investigated in detail. Mycelium from infected cotyledons became established in the cortical cells of the stem without any apparent effect upon them, and remained localized, in the immediate area that had been entered originally, until the time of flowering. It then resumed growth and penetrated cells in the stem, petioles, and leaves without the development of disease symptoms. When pods started to develop after fertilization, mycelium was found in the cells of the pod. These pods and the developing seeds also had no evidence of disease until maturity.

The effect of some leaf diseases on grain yield in corn. ULLSTRUP, ARNOLD J. Studies were made of the effect of northern corn leaf blight, caused by *Helminthosporium turcicum*, and of Helminthosporium leaf spot, caused by *H. carbonum* race I, on grain yields of susceptible and of resistant single crosses. Yields produced under severe, artificially induced epiphytotics of these diseases were compared with the performance of the same materials under essentially the same environment but in the absence of the diseases. Northern corn leaf blight reduced the yield of susceptible single crosses from 40 to 68 per cent. Yield reductions sustained by resistant single crosses ranged from 1.8 to 18 per cent. Helminthosporium leaf spot reduced grain yield in a susceptible single cross 79 per cent. In the absence of this disease the difference in yield between the resistant and the susceptible single cross was nonsignificant.

Effect of temperature on symptom expression of a virus disease of cucumber transmitted mechanically from sour cherry. VARNEY, E. H., AND J. DUAIN MOORE. During March and April, 1950, isolates A and B of a virus disease of cucumber transmitted mechanically from sour cherry were compared on cucumber seedlings at various temperatures. All cucumber seedlings were grown before inoculation on open greenhouse benches at 65°–70° F. After inoculation, plants were held at one of the following approximately constant temperatures: 61°, 70°, 75°, 80°, 85°, 90°, 95° F. Equal numbers of uniform plants were inoculated with each isolate and placed at each temperature. Initial symptoms incited by the two isolates were similar. Both incited chlorotic circular lesions within 1½ to 2 days at 75° and above, 3 days at 70°, and 4 days at 61° F. Few lesions developed at the high and the low temperatures but many at 80° and 85° F. In 7 days, isolate A killed all plants at 61° and most of those at 70° F.; B did not develop systemically at 61°, although it killed growing points at 70°; all growing points were destroyed by isolate A at 75°, 80°, and 85°, whereas B killed only a few at 75° and none at the higher temperatures. At 90° and 95° F. both isolates were mildly systemic in only a few cases.

Cycloheximide, an antibiotic effective against turf diseases. VAUGHN, JOHN R. Cycloheximide, at 200 p.p.m., was sprayed at 2-week intervals throughout the season on golf-green plots at six locations in Michigan, in comparison with two standard turf fungicides. Counts of dollar spot (*Sclerotinia homeocarpa*), which was prevalent at five locations in both spring and fall, were consistently lower in cycloheximide-treated plots than in those treated with other materials. Melting out (*Helminthosporium* sp.), which was severe at one location, was completely checked by cycloheximide but not by the other fungicides. In cycloheximide-treated plots no phytotoxicity was observed on any of the several strains of bent grass.

Gradients of potato late blight observed around artificially inoculated plants. WAGGONER, P. E. Two potato plots having straight rows and a third having concentric circular rows were grown in three different environments. Plants in the center of each plot were inoculated with *Phytophthora infestans*. Continuous meteorological records were kept. The percentages of living, mature leaflets with active lesions were estimated in all plots at approximately weekly periods. The estimates were based upon a sample of ten upper and ten lower leaflets on two randomly selected stems per 1-yd. length of straight row and 22½° arc of circular row. The data showed that late-blight lesions appeared first on the lower leaflets and later became more prevalent on the upper leaflets. The gradient changed with time: the rapid initial decrease with distance from the source of inoculum in the percentage of leaflets with lesions became progressively smaller. Expressions for disease as a function of distance and at least two other parameters have been proposed. Values for parameters having physical meaning were obtained by fitting to the data. These were compared to independent estimates of the parameters. All of these results are useful in inferring the history of epiphytotics from observations of disease gradients.

Plant part temperatures influencing the epiphytology of potato and tomato late blight. WAGGONER, P. E., AND R. H. SHAW. The influence of shading, cloud cover, time of day, species, spacing in the row, and plant part upon the temperature of that part was determined. The temperatures were measured with 30-gauge copper-constantin thermocouples and a recording potentiometer capable of traveling 150° F. in 5 sec. Shaded leaflets were more than 10° F. cooler than exposed leaflets. Sky cover decreased the difference between exposed upper and shaded lower leaflets. Little difference between potato and tomato plants and between open and closed plantings was found. Leaflet, petiole, and upper stem temperatures were approximately equal; lower stem temperatures were as much as 5° F. cooler. Therefore, upper leaflet temperatures can be expected to be higher and generally less favorable for late-blight development than lower leaflet or instrument shelter temperatures during clear days; furthermore, evaporation could be increased on

the upper leaflets by the large difference between air and evaporating surface temperatures. In cloudy weather and at night the temperatures will not differ greatly. Evidently conduction of heat down the stem to the cooler subsoil cools the lower stem and could enable *Phytophthora infestans* to survive hot weather.

Effect of fungicides on the development of red rot of sugar cane. WAHID, M. A., R. J. STEIB, AND S. J. P. CHILTON. Latent infections of *Colletotrichum falcatum* occurring in the bud and leaf-scar tissues of sugar cane led to attempts to reduce this infection by dusting growing cane with fungicides. A 10 per cent dust containing either Zerlate, Fermate, or Parzate was used. After the first year, Parzate only was used. The dust was applied to growing canes of the variety Co. 290 each week for from 5 to 10 dustings, beginning when joints appeared. Tissues from the leaf scars and buds were plated on oatmeal agar to determine presence of the fungus. Of 805 nodes of undusted canes, the fungus was obtained from 63 per cent of the leaf scars and 66 per cent of the buds. Of 850 nodes from cane dusted with Parzate, the fungus was obtained from 25 per cent of the leaf scars and 29 per cent of the buds. Of over 200 nodes with each fungicide, the same reductions were obtained where Zerlate and Fermate were used. Stalks of cane were collected from four of these tests and held at 70° F. After 30–40 days, the fungus had gone into the stalks of 49 per cent of the undusted joints; the fungus was present in 30 per cent of the dusted canes.

Progress in the development of clubroot-resistant cabbage. WALKER, J. C., AND R. H. LARSON. From backcrosses of resistant kale-cabbage rogues with four yellows-resistant varieties of cabbage, selections were made for clubroot resistance and type in succeeding generations. Highly resistant lines of acceptable type were secured in the F_5 generation in the cross with Wisconsin Ballhead. A second backcross was necessary in the case of Marion Market, Resistant Charleston Wakefield, and All Head Select. Susceptibility was completely dominant in crosses between highly resistant and highly susceptible parents. Ten to 20 per cent of the individuals in the F_2 were resistant, indicating polygenic inheritance. A line of Wisconsin Ballhead resistant to clubroot and yellows is ready for increase. Further selection is necessary in the other lines. These results show that clubroot resistance can be combined with yellows resistance in any desired cabbage type.

Development of a scab-resistant pickling cucumber for Wisconsin. WALKER, J. C., AND A. B. WILES. Starting with Maine 2 variety as a source of resistance, a backcrossing program with National Pickling, a standard variety in general use, was initiated in 1947. By means of greenhouse propagation, three generations per year were secured. Five successive backcrosses were made, in each of which susceptible plants were eliminated by inoculation in the greenhouse at 19° C. when plants were in the first-leaf stage. After the fifth backcross two successive self generations yielded homozygous resistant lines which were tested in the field in 1950. Certain of these lines were sufficiently close to the National Pickling to warrant increase for release for commercial use.

Response of the tomato and potato in field plots inoculated with Phytophthora infestans. WALLIN, J. R. A potato isolate of *Phytophthora infestans* was used to inoculate potatoes in interplanted tomato and potato plots in Iowa and Indiana. Only potatoes were inoculated at Clear Lake, Iowa, and Lafayette, Indiana, to determine rapidity and extent of spread of the pathogen to potatoes and tomatoes. At Clear Lake, potatoes were severely blighted 6 weeks after inoculation; at 9 weeks, vines were defoliated. No blight symptoms were found on tomatoes during the season. At Wanatah, Indiana, 100 per cent of the potatoes were blighted 5 weeks after inoculation. No lesions were found on tomatoes. During maximum spread on potatoes no lesions were found on tomatoes. Later, when 75 per cent of the potatoes were defoliated, a few lesions appeared on tomatoes. At Lafayette, 5 weeks after inoculation, moderate infection occurred on potatoes but only a trace on tomatoes. The tomato blight may have been caused by spore drift from near-by tomato fields. Significantly, the potato isolate failed to become established on tomatoes during the season at Clear Lake and for 5 weeks at Wanatah, although adjoining potato plants became severely blighted. If tomato blight in Indiana plots was caused by the potato isolate, its development was definitely slower than on potatoes.

Forecasting tomato and potato late blight in the north-central region. WALLIN, J. R. During the 1950 growing season, eight hygrothermographs were distributed among five potato fields, one tomato field, and two plots which had been inoculated with *Phytophthora infestans.* In the analysis of the hygrothermograph charts, conditions were considered favorable for sporulation, spore germination, and host reinfection by the pathogen if the temperature and relative humidity for 10 or more consecutive hours were 70° F. or less and 95 per cent or more, respectively. Usually these conditions prevailed at night; hence, the frequency of favorable nights was used to indicate the development of *P. in-*

festans. In the three predictions made based upon the above conditions, late blight development was forecast for five areas of the region, including the inoculated plots. The disease occurred in all five areas. Charts from two areas indicated that the daily humidities were too low to favor infection cycles of *P. infestans.* No late blight was reported in these areas. The results indicate that hygrothermographs placed in the field yielded rather accurate information on the temperature-humidity conditions associated with the cyclic development of *P. infestans* in the field, and that they can be successfully used as a basis for forecasting late-blight development.

Relation of host nutrition to the development of bacterial wilt of cucumber and fusarial wilt of watermelon. WEI, C. T., AND R. P. SCHEFFER. The effects of host mineral nutrition on development of bacterial wilt of cucumber (*Erwinia tracheiphila* (E. F. Sm.) Bergey et al.) and fusarial wilt of watermelon (*Fusarium oxysporum* f. *niveum* (E. F. Sm.) Snyder & Hansen) were studied in sand culture. Five concentrations of Hoagland's solution, low and high levels of N, P, and K, and long- and short-day exposures were used. Cucumber wilt increased in severity with increase in concentration of nutrient supply. Disease development was favored by low N and high K; it decreased in high N. Disease indices of short-day plants were lower than those of long-day plants only when experiments were performed during winter months. Fusarial wilt of watermelon, on the other hand, was favored by low concentrations of Hoagland's solution and by high K. Disease ratings were slightly higher in high P and high N than in low P and low N, respectively. Day length had no significant effect. No correlation was found between fresh or dry weights of host plants and development of the two diseases.

Tree defoliation for control of Omphalia disease of coffee. WELLMAN, FREDERICK L. Recently published studies on *Omphalia flavida* Maubl. & Rangel, cause of the most serious coffee disease in the western hemisphere, have shown that it is mostly dependent for dissemination on relatively heavy infection bodies. These are macroscopic, not sticky, detached from lesions with difficulty when dry but quickly loosened when wet, and, being disseminated in rainwater droplets, progress slowly from tree to tree. Use of Bordeaux spray has long been advised as a control but is economically and physically impossible to employ properly under usual coffee-plantation conditions. Defoliation treatment of severely diseased trees was tested in 15 plots, under ideal conditions for disease in Turrialba, Costa Rica. Removed foliage was left to decay on the ground. New healthy leaves developed in six weeks and remained healthy, accompanied by renewed tree vigor. In treated plots fruits and flowers increased up to more than 1000 per cent over those in untreated diseased trees. With proper vigilance, extensive defoliation should never have to be repeated.

The use of C^{14}-labeled sucrose for "tagging" fungus mycelium. WHEELER, H. E. C^{14}-labeled sucrose has been used to study sexual reaction between strains of the fungus *Glomerella.* A small drop of a medium containing 1 or 2 per cent C^{14}-labeled sucrose with a specific activity of 0.5 microcuries per mg. as the sole carbon source was spread over part of the upper surface of a cover slip placed on one side of a Petri dish containing oatmeal agar. Matings were then made by planting one strain of the fungus on the agar drop containing C^{14}-labeled sucrose, a second strain being planted on the opposite side of the Petri dish. The mycelia of the two strains were allowed to grow together; after a line of perithecia had formed at the point of contact, the cultures were dried and radioautographs made. Since the mycelium produced by the strain planted on the agar drops containing C^{14}-labeled sucrose was radioactive, it was possible to determine which of the two mated strains produced the perithecial fundaments. The fact that fungus mycelium can be effectively "tagged" by this technique suggests that this isotope may be very useful in attacking a number of problems in the field of plant pathology. The C^{14}-labeled sucrose used in these experiments was supplied by G. R. Noggle of the Biology Division of the Oak Ridge National Laboratory.

Studies on Pseudomonas lachrymans in cucumber. WILES, A. B. Angular leaf spot in some years is a destructive disease of cucumber in the pickle-growing area of northeastern Wisconsin. In the greenhouse high humidity and temperatures between 24° and 28° C. are optimum for disease development. While the association of *Pseudomonas lachrymans* (E. F. Sm. & Bryan) Carsner with cucumber seed has long been recognized, the relation of the organism to the seed and other host tissues needed further study. In leaf, stem, and fruit the bacteria are found primarily in the parenchymatous tissues, where they are initially intercellular becoming intracellular as cell walls rupture. In infected fruits the organism is found within the xylem elements of the mesocarp. It is also found in the tissue immediately surrounding the developing seed as well as within the placentae and the funiculi. Infected seeds may harbor the pathogen on the exterior of the seed coat, beneath the seed coat, or in both locations.

Comparative performance of 40 fungicidal formulations in the control of a severe outbreak of tomato late blight. WILSON, J. D. That late blight was severe in this experiment is shown by the fact that 88 per cent of the fruits on the untreated checks were diseased. The best treatment reduced this to 2 per cent. When the various formulations were ranked on the basis of the percentage of diseased fruits, the first 11 were found to contain copper, either used alone or in combination with various other materials. The next three places were taken by various ethylene bis dithiocarbamates. Near the bottom of the list in the control of late blight were a number of dimethyl dithiocarbamates. A fixed copper plus an adhesive gave the lowest percentage of diseased fruits, Bordeaux was fifth, and two copper compounds of metallic nature were ninth and tenth. A zineb was thirteenth. A tank mixture of ziram and a fixed copper was somewhat more effective than the same two materials used in an alternating schedule. This suggests again that such combinations may deserve further study.

Rust infection increases invasiveness of tobacco-mosaic virus in bean. YARWOOD, C. E. When Pinto and some other varieties of beans were inoculated with the uredial stage of *Uromyces phaseoli* and with tobacco-mosaic virus (TMV) within 4 days of each other, necrotic rings formed around some of the rust pustules, indicating that the TMV had increased without host necrosis till it reached the periphery of the rust colony. The area of the TMV-infected rust pustules was about 50 times the area of TMV local lesions on nonrusted tissue. Inoculations of *Nicotiana glutinosa* with juice from comparable areas of TMV-inoculated rusted and nonrusted leaves indicated that the virus was about 50 times as concentrated in the rusted as in the nonrusted leaves. Rust sporulation was less in TMV-infected than in normal rust pustules. Inoculation of beans with urediospores from TMV-infected pustules gave normal pustules. Inoculations of *N. glutinosa* with extract from crushed spores from TMV-infected pustules gave no TMV local lesions. Spore extract added to TMV reduced its infectivity on *N. glutinosa*. When the rust mycelium in a heavily infected leaf was killed by pressing the leaf between the fingers without seriously injuring it, the number of TMV lesions was greater than when the rust was not so killed.

Inoculation methods for tobacco-mosaic virus on bean. YARWOOD, C. E. When Pinto beans were inoculated with tobacco-mosaic virus in studies of the TMV-bean rust association, the number of local lesions on nonrusted leaves varied from 0 to 30 per cm.^{2} in different trials. In no case was TMV infection of all separated rust pustules attained. When individual leaves or entire plants were held in dark or light moist chambers before or after inoculation, the TMV lesions were generally more numerous and sometimes larger than on leaves held in the normal greenhouse environment. Inoculations, with or without carborundum, by brushing dry leaves with a dry brush in which an extract from tobacco mosaic had dried sometimes gave more lesions than did the conventional wet inoculation. With detached leaves more lesions formed at 31° than at 25° or 19° C., and the lesions were much larger for leaves on water than for leaves on 8 per cent sucrose.

Reversibility of copper toxicity to conidia of Sclerotinia fructicola. YODER, DONALD M. The toxic effect of $CuSO_4$ solutions on respiration and germination of conidia of *Sclerotinia fructicola* is partially or completely reversed by the addition of solutions of sulfhydryl-containing compounds. The compounds successfully employed for this purpose were cysteine, reduced glutathione, 2,3-dimercaptopropanol, thioacetic acid, and sodium thioglycollate. The completeness of the reversal was found to be dependent on the molar ratio of antidote to $CuSO_4$, the length of time elapsing between the addition of Cu solution and the addition of antidote, and the type of antidote. The reversibility of Cu toxicity was demonstrated, using not only conidia of *Sclerotinia fructicola* but also conidia of *Botrytis gladiolorum*, *Helminthosporium victoriae*, and *Fusarium oxysporum* f. *gladioli*; and sporidia of *Ustilago zeae*. It is suggested that these tests are evidence for a mode of action of Cu ions involving inhibition of respiratory enzymes. The mechanism of the reversal is believed to involve withdrawal of the metal ions from the site of inhibition in the enzyme protein by mercaptide formation with antidote molecules. The mercaptides formed are so weakly dissociated that effective removal of the heavy metal ions from the enzyme surface is accomplished.

TOLERANCE OF WEDGEWOOD IRIS BULBS TO A HOT-WATER–FORMALIN TREATMENT[1]

WILBUR D. COURTNEY[2] AND CHARLES J. GOULD[3]

(Accepted for publication July 25, 1950)

Bulb or stem nematodes were first reported infecting iris bulbs by Van Poeteren (6) during 1925, in Europe. The first appearance in the United States was in Virginia in 1928, as noted by Steiner and Buhrer (5); by July 1932 they had observed 38 infected lots, 23 imported and 15 domestic-grown. These included varieties of Dutch, English, Moroccan, and Spanish types of iris. Newton, Hastings, and Bosher (4) found this nematode infection in British Columbia during 1934, and by 1937 it was present in many varieties of Dutch iris. In western Washington, Courtney[4] found shipments of imported iris infected with bulb or stem nematodes in 1934.

This bulb pest, brought to Washington with early imports, apparently failed to establish itself until recent years; at first in some of the San Juan Islands, and finally, during World War II, throughout western Washington and Oregon. The bulbous iris industry of the Pacific Northwest, with a gross income of about $1,000,000, was soon showing an increasing annual loss. Bulb losses ranged from a trace up to 60 per cent, or an average of 20 per cent, due to nematode infections.[5] Hot-water treatments of 50 min. at 110°–112° F. as suggested by Staniland, for 60 min. at 110°–112° F. as outlined by Hastings et al. (3), or 60 min. at 110°–112° F. following a 2½-hr. presoaking at 70°–80° F. developed by Weiss and reported by Hastings et al. (3), failed to control nematode infections in Oregon and Washington. The earlier investigations will be discussed in a later report. Present data indicate that a 3-hr. duration of 110°–110.5° F., with 1 pt. commercial formaldehyde solution U.S.P. added for each 25 gal. of treating bath, is necessary for complete nematode kill under western Washington conditions. Results of tests have demonstrated that iris bulbs can be successfully treated in hot-water-formalin for nematode control in western Washington.

MATERIALS AND METHODS

Iris bulbs. These tests were confined to Wedgewood iris and included 8,000 bulbs each of slabs (5–6 cm. in circumference), rounds (7–8 cm.), and

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[4] Unpublished annual report for 1934.

[5] In Oregon and Washington State Departments of Agriculture Surveys, March 1949. Unpublished.

mothers (10–12 cm.). (Mothers are iris bulbs which have bloomed and become somewhat flattened in shape. Rounds are bulbs which have not yet bloomed. Slabs are small bulblets produced at the base of round and mother bulbs.) They were dug on four different dates, then cleaned, graded, and treated 2, 4, and 6 weeks after digging. They were then air dried and placed in common shed storage until planted in the field during early October, 1946. A very early digging was made on June 19, with treatments of the lot on July 3, 17, and 31. An early digging on July 3 was followed by treatment on July 17 and 31 and August 14. The normal digging was on July 17 and treating was on July 31, August 14 and 28. A late digging on July 31 was followed by treatment on August 14 and 28 and September 11.

Treatment. The treatment consisted of immersion in water at 110° F. for 4 hr., with 1 pt. commercial formaldehyde solution added for each 25 gal. water. This is the standard hot-water-formalin treatment for narcissus bulbs (1) and was used in preference to the 3-hr. duration which Courtney previously had found satisfactory for nematode kill in iris. The purpose of the prolonged duration was to accentuate possible plant injury resulting from treatment.

Plot arrangement. Five replications of 100 bulbs each per treatment were planted in the field, as randomized blocks in a factorial design, and handled as commercial stock.

RESULTS

Each lot was judged at various times during the growing and harvesting season of 1947 from the standpoint of several criteria. The most important of these were: 1) Early emergence, as determined by the number of plants emerged on February 3, 1947. An excessive early plant growth might increase crop damage from frost injury. 2) Total number of plants, as determined by the total number of plant clumps harvested in July 1947. 3) Total bulb weight, as determined by the total harvested weight, in grams, of commercial-sized bulbs of 5 cm. in circumference or more. 4) Average weight of bulb clumps harvested, used as a measure of plant vigor and productiveness. 5) Number of slabs per plant used as an index of bulb division that would be detrimental if excessive. 6) Ratio of rounds to mothers, as determined by the number of rounds divided by the number of mothers, for each lot harvested. An excessive number of mothers would indicate a premature division of rounds and a resultant financial loss, as salable bulbs for forcing are rounds of 10–11 cm. or more. 7) Number of dormant bulbs, as determined by the number of bulbs harvested which had not developed foliage during the growing season. Such bulbs are usually sound, but are undersized as compared with others of the same age.

The most indicative of the above criteria is that of total bulb weight harvested. Detailed data are in table 1.

TABLE 1.—*Effect of digging dates and curing periods of iris bulbs on their ability to withstand the 4-hr. hot-water-formalin treatment as measured by means of total bulb weight harvested (grams)*

Bulb type and curing period	Digging dates				
	Very early	Early	Normal	Late	Average
Slabs					
Check (untreated)	179.4*	404.8	516.8	488.0	397.2**
2 weeks	68.6	154.4	292.8	267.6	195.8
4 do	184.4	377.4	346.6	400.8	327.3
6 do	192.2	455.2	371.8	363.8	345.8
Average	156.2**	348.0	382.0	380.0	
Rounds					
Check (untreated)	825.0*	1,262.4	1,376.6	1,621.6	1,271.4
2 weeks	401.0	1,207.2	1,472.8	1,391.8	1,118.2
4 do	865.0	1,561.8	1,526.6	1,291.6	1,311.2
6 do	826.2	1,883.4	1,065.2	336.2	1,027.8
Average	729.3**	1,478.7	1,360.3	1,160.3	
Mothers					
Check (untreated)	1,246.0*	2,828.0	2,474.8	2,903.0	2,362.9
2 weeks	1,093.4	2,372.6	1,989.4	2,665.0	2,030.1
4 do	1,448.0	2,875.0	2,306.2	1,936.2	2,141.3
6 do	1,290.2	2,561.6	1,971.8	374.6	1,549.5
Average	1,269.4**	2,659.3	2,185.5	1,969.7	

		Slabs	Rounds	Mothers
* L.S.D., individual treatments:	19 : 1	101.3	271.1	454.9
	99 : 1	134.7	360.6	605.0
** L.S.D., digging dates and curing periods:	19 : 1	50.6	135.6	227.5
	99 : 1	67.3	180.4	302.5

The data for slabs indicate that: 1) The average weight of all untreated sets was significantly higher than that of the treated sets. The weights of sets treated after 4 and 6 weeks of curing were significantly higher than that of the 2-week set. 2) The average weight of all very early digging trials was much less than that of other dates. There were no significant differences between yields from early, normal, and late digging. 3) With very early and early digging no significant differences existed between the untreated checks and treatments after 4 and 6 weeks of curing. Treatments after 2 weeks of curing, however, were very harmful. 4) All treatments were very harmful when applied to bulbs lifted on the normal digging date. The length of the curing period exerted no significant influence. 5) With late digging, treatment after 2 and 6 weeks of curing was harmful as compared with the check.

The data for rounds indicate that: 1) The average yields of untreated bulbs and those treated after 4 weeks of curing were significantly greater than those from bulbs treated after 2 and 6 weeks of curing. 2) The average weight of rounds in the early-dug trials was significantly greater than the yields for very early and late dates. 3) With very early digging no significant differences existed between the checks and treatments after 4 and

6 weeks of curing. Treatments after 2 weeks of curing were very harmful. 4) With early digging, treatments after 6 weeks of curing gave best results, followed by 4 weeks of curing and check. Treatments after 2 weeks of curing were the poorest. 5) With normal digging no significant differences existed between the check and treatments after 2 and 4 weeks of curing. The 6-week curing period was very harmful. 6) With late digging, treatment after 4 weeks of curing was harmful. It was very harmful after 6 weeks. 7) The largest yield of the untreated sets was from bulbs dug on the late date. The largest yield of the treated sets was in the early-dug set treated after 6 weeks, followed by early-dug—4 weeks, normally dug—4 weeks, and normally dug—2 weeks.

The data for mother bulbs indicate that: 1) The average weight of all trials cured for different periods showed best results in the untreated checks and the 4 weeks of curing. The yields from the 2- and 6-week sets were very much less than from the untreated set. 2) The average weight of all very early digging trials was less than that of the other dates. The early digging was best, followed by normal and late dates. 3) With very early digging, no significant differences existed between the treatments and checks. 4) With early digging, treatments after 2 weeks of curing were harmful as compared with the check and 4-week period. There were no other significant differences. 5) With normal digging, the 4-week curing periods were not significantly different from the checks. The 2- and 6-week periods were harmful as compared with the check. 6) With late digging, the yields from checks and 2-week-curing-period sets did not differ statistically, but those from 4- and 6-week periods were greatly reduced.

Table 2 shows favorable and detrimental trends, along with significant results, at the 5 and 1 per cent levels. While these trends do not exert significant weight, their consistent expression is of value in selecting a treatment program where so many variations occur, because of the narrow margin of safety between plant injury and pest control.

DISCUSSION

The detailed results as expressed in table 1 are quite comparable with the trends of expression in table 2. In general these indications may be summarized as follows: 1) While very early digging is detrimental to iris plants, early and normal digging tend to result in better crops, if treatments of iris stock are to be made. The crop should be dug in early season and treated before late August in the Puyallup area. 2) Late digging is not so detrimental to untreated bulbs as the very early digging, but very severe injury is certain to result from treatment of late-dug stock, especially after mid-August. 3) Slabs, rounds, and mothers may all be successfully treated in hot-water-formalin, if done in proper season, but rounds (7–8 cm.) gave the best results. 4) Results enumerated above were secured under conditions existing in the Puyallup Valley, Washington, during the summer of 1946 and the growth period of 1946–1947. These records, together with

TABLE 2.—*Summary of results of iris bulbs dug at four intervals and treated for 4 hr. in hot-water-formalin 2, 4, and 6 weeks afterwards, as compared with similar untreated sets. Treated in 1946 and harvested in 1947*

Type of bulb treated and measurement	Digging dates											
	Very early			Early			Normal			Late		
	2	4	6	2	4	6	2	4	6	2	4	6
Slabs												
Emergence	0−	0−	0−	0−	0−	0−	−−	−−	−−	−	0−	−−
Total plants	−−	0−	0−	−−	0−	0+	−−	−	0−	−−	−	0−
Total bulb weight	−	0+	0+	−−	0−	0+	−−	−−	−−	−−	0−	−
Weight per plant	−−	0−	0+	−−	0+	0−	−−	−	−−	0−	0−	−
Slabs per plant	0	0	0	0	0	0	0	0	0	0	0	0
Ratio of rounds	0	0	0	0	0	0	0	0	0	0	0	0
Dormant bulbs	0+	0	0+	0−	0−	0+	0−	0−	−	0−	0−	+
Rounds												
Emergence	0−	0+	0−	++	0+	0+	0+	0+	−−	0−	−−	−−
Total plants	−	0−	0−	0+	0+	++	0+	0+	0−	−	0−	−−
Total bulb weight	−−	0+	0+	0−	+	++	0+	0+	−	−	−	−−
Weight per plant	−−	0+	0+	0−	0+	++	0+	0+	−−	0+	0−	−−
Slabs per plant	−	0+	0+	0−	0+	++	0	0+	0−	0−	0−	−−
Ratio of rounds	0−	0−	0−	0+	0+	0+	−	0+	0−	−−	−	−−
Dormant bulbs	0−	0−	0−	0+	0+	0+	0−	0−	0−	0−	0−	++
Mothers												
Emergence	0+	0+	0+	0+	0+	−	0−	0−	−−	0−	−−	−−
Total plants	0−	0+	0−	0−	0−	0−	0−	0−	0−	0−	−−	−−
Total bulb weight	0−	0+	0+	−	0+	0−	−	0−	−	0−	−−	−−
Weight per plant	0+	0+	0+	0−	0+	0+	0−	0−	0+	0+	0+	−−
Slabs per plant	0+	0−	0+	0−	0+	0+	0−	0+	0+	0+	0+	0−
Ratio of rounds	0−	0−	0−	−−	−−	−−	−	0−	0−	0−	0−	+
Dormant bulbs	0	0	0	0	0	0	0	0	0	0	0	0

0 = no difference as compared to the untreated set; 0− and 0+ = minus or plus differences less than 5 per cent levels; − and −− = minus differences of 5 and 1 per cent respectively; + and ++ = plus differences of 5 and 1 per cent respectively.

observations on extended commercial treatments of iris bulbs in hot-water-formalin indicate that the ability of these bulbs to withstand such a treatment is dependent upon the maturity of the bulbs at the time of treatment. Climate, stocks, soils, and storage conditions undoubtedly affect maturity in iris bulbs, rather than curing time alone. The term "maturity" as used in this paper is construed by the writers to mean the most complete resting phase of the iris bulb. 5) Commercial treatments based on these experiments are rapidly becoming a part of the production program of bulbous iris growers in the Pacific Northwest. It is estimated that at least one-half of the iris bulbs planted in this area during the 1948 season were treated, with an estimated annual saving of $100,000 to iris growers of Oregon and Washington.

SUMMARY

The experimental tests of 1946–1947 have demonstrated that iris bulbs can be safely treated in hot-water-formalin for nematode control. A 4-hr. duration was used in these tests to increase and thus make possible a better

evaluation of plant injury, although 3 hr. appears to be sufficient for this purpose under conditions existing in the Puyallup Valley, Washington.

The success of the treatment was determined by comparing lots treated 2, 4, and 6 weeks after digging (500 bulbs each), with the untreated checks, as to emergence, total number of bulbs harvested, total weight of bulbs, weight of bulbs per plant, number of slabs per plant, ratio of rounds to mothers, and number of dormant bulbs.

While mother bulbs and slabs may be successfully treated, rounds (7–8 cm.) appeared to react most favorably to the hot-water-formalin treatment. For best results the bulbs must be dug as early as possible after danger of shrinkage is past, and treated before late August in the Puyallup area.

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DUTCH ELM DISEASE IN YOUNG ELM SEEDLINGS[1]

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The difficulty of successfully inoculating 1- and 2-year-old elm seedlings with the Dutch elm disease fungus, *Ceratostomella ulmi* (Schwarz) Buisman, has seriously impeded progress on control and chemotherapy studies. Heretofore such investigations, limited to older trees growing in the field, have been confined to the normal growing season. The use of young elm seedlings is advantageous because their availability in large numbers expedites a greater number of experiments and permits greenhouse investigations where conditions can be better controlled.

Various investigators refer to infection of small trees by the Dutch elm disease fungus. May (9) reported that in Europe both nursery seedlings and large mature trees have been found infected. Clinton and McCormick (5) referred to the inoculation experiments of "seedling elms" by Christine Buisman (3, 4). In their extensive inoculation investigations, Zentmyer, Horsfall, and Wallace (10) employed more than 2,000 American elms growing in nursery rows. However, none of these investigators have made reference to the use of 1- and 2-year-old seedling elms.

EXPERIMENTATION

The initial problem was to determine whether disease could develop in young seedlings as readily as in older field trees.

Agar-disc method of inoculation. In May 1946 two very pathogenic isolates of *Ceratostomella ulmi*, "black" strain and No. 124[3], were used to inoculate 30 two-year-old potted seedling elms and 30 six-year-old field elms. A 5-mm. agar disc, from a 3-week-old heavily sporulating culture, was inserted into a vertical wound at the base of each tree. On the basis of Banfield's work (2), wounds were made so as to insure severance of vessels. Sterile medium was inserted in the control plants. Ten seedlings and 10 field trees were inoculated with each isolate, and 10 control plants were provided.

The black strain wilted 70 per cent of the field trees and caused vascular discoloration in 80 per cent. It wilted only 10 per cent of the seedlings and discolored 50 per cent. Isolate No. 124 wilted 60 per cent of the field trees and discolored 70 per cent; none of the seedlings were wilted by this isolate and only 30 per cent had vascular discoloration. Seedlings are not so readily infected as older trees.

Spore suspension inoculation tests. Banfield (1) and Zentmyer et al. (10) demonstrated that a spore suspension inoculum resulted in better

[1] Contribution No. 759 of the Rhode Island Agricultural Experiment Station, in cooperation with Bartlett Tree Research Laboratory.

[2] The authors wish to express their appreciation for the technical assistance of Mary-Elizabeth Davies.

[3] Isolated originally by Dr. K. G. Parker near Yonkers, N. Y., and by Mr. Nestor E. Caroselli in Stamford, Connecticut, respectively.

disease development than agar-disc inoculations. In June 1947 a series of 10 seedlings and trees were wounded and inoculated with spore suspensions of the isolates; similar controls were wounded and inoculated with washings of sterile medium. In field trees the wound was made under 3 cc. of a spore suspension held in a saucer-shaped beeswax collar.

There was no difference in the number of diseased seedlings inoculated by either the spore-suspension or agar-disc method. In field trees, however, results of Zentmyer et al. (10) were corroborated and pathogen establishment was better with the spore suspension method. Black strain wilted and discolored 100 per cent of the trees; No. 124 wilted 80 per cent and discolored 90 per cent.

EFFECT OF DARKNESS ON PREDISPOSING SEEDLINGS TO DISEASE

The apparent resistance of seedlings to wilting voids their use for experimental work unless some means of increasing susceptibility is perfected. On the basis of physiological studies, Feldman, Caroselli, and Howard (6, 7) formulated the hypothesis that a physiological change in young trees, subjected to certain periods of total darkness, might result in formation of certain host products favorable for pathogen growth and greater toxin production, which, in turn, might injure the host more and be reflected in more pronounced wilt symptoms.

TABLE 1.—*Incidence of wilt symptoms in elm seedlings subjected to various periods of darkness*

Group and treatment[a]	Percentage of trees	
	Wilted	With vascular discoloration
A. Dark 5 days—Inoculation—Dark 7 days	0	10
B. Daylight[b]—Inoculation—Dark 7 days	0	30
C. Daylight—Inoculation—Dark 5 days	30	30
D. Dark 5 days—Inoculation—Daylight	100	100
E. Daylight—Inoculation—Daylight	0	30

[a] Noninoculated trees receiving comparable light and dark exposures had no wilt or vascular discoloration, nor were they defoliated.
[b] Treatment consisted of 9-hr. daylight.

Two-year-old elm seedlings in full leaf were subjected to varying exposures of darkness before and after inoculation (Table 1). The inoculum was prepared from a culture of the black strain and contained approximately 1,000,000 spores per cc. The tube method of inoculation used (Fig. 1) consisted of a 1½-in. length of ⅜-in. gum rubber having a ½-in. slit at one end. The slit end was placed over the area to be inoculated and secured by a small length of wire. The first step of inoculation was accomplished by pulling down the rubber tube to a nearly horizontal position, making a small pocket adjacent to the tree; in this pocket a small quantity of the spore suspension was placed. A cut was made with a sharp knife into the xylem tissue beneath the spore suspension, so as to insure a rapid intake of the inoculum through the severed vessels. To maintain a liquid-proof seal, the base of the rubber tube was cemented to the tree with plastic putty. One cc. of the spore

suspension was then placed in the rubber tube in order to maintain an adequate supply of inoculum.

Fifty 2-year-old seedlings were divided into five groups of 10 plants and were treated as follows (Table 1): Group A trees were subjected to total darkness for 5 days, inoculated, and kept in total darkness for an additional 7 days. Group B trees remained in 9-hr. daylight until they were inoculated, after which they were kept in total darkness for 7 days. Group C trees were treated the same as group B except for being kept in total darkness for 5 days after inoculation. Group D trees were placed in total darkness for 5 days, inoculated and kept in 9-hr. daylight. Group

FIG. 1. Rubber-tube method of inoculation. Introduction of a heavy spore suspension by means of a rubber tube attached to the seedling.

E trees were kept in 9-hr. daylight before and after inoculation. Seedlings used as checks for each group were inoculated with a washing from potato-dextrose agar and were subjected to the same light and dark treatment. The minimum period of 5 days total darkness was selected since preliminary experiments showed little or no pathogen establishment in seedlings subjected to shorter periods.

Wilt appeared on the affected plants about 1 to 3 weeks after inoculation (Fig. 2). Periodic observations on the progress of wilt and the presence of vascular discoloration were made. As shown in table 1, the greatest number of seedlings having wilt and vascular discoloration were those exposed to total darkness for 5 days prior to inoculation (group D). These seedlings were completely defoliated within 1 month after inoculation.

Eighty per cent of the seedlings in group D had wilt symptoms within 3 weeks after a new set of leaves appeared. A striking difference in the amount of disease is readily noted by comparing groups C and D. Better disease resulted in group D where time of inoculation was after dark treatment as compared to group C which was inoculated before treatment.

In the agar-disc and spore-suspension tests, 50 to 60 per cent of the seedlings had vascular discoloration; a maximum of only 10 per cent of the seedlings manifested wilt symptoms. These tests are comparable to those of group E in which only 30 per cent of the trees had vascular discoloration and none had wilt symptoms. No reasonable explanation can be given for the difference in vascular discoloration noted. Subsequent

FIG. 2. Foliage symptoms. Seedling on the left was placed in dark for 5 days, inoculated, and placed in light. Seedling on the right received the same dark and light treatment but was inoculated with a washing from sterile medium.

tests, however, using the same light and dark treatments yielded results comparable to those shown in table 1.

Since toxin production is favored by the presence of asparagine (10), it was believed that the addition of asparagine to the inoculum might increase the disease. An inoculum-asparagine treatment of seedlings having received the same dark treatments as group A, however, did not increase the incidence of disease.

Likewise, no appreciable increase in disease was noted in trees inoculated with spores suspended in culture filtrate in which the fungus had grown (toxin complex).

DISCUSSION

In the first seedling inoculation experiments it was found that, although vascular discoloration was evident in about 50 per cent of the inoculated trees, only 10 per cent or less had wilt symptoms. In order to evaluate the

fungicidal and toxin-inactivating properties of chemicals on seedlings, it was necessary to produce the same syndrome on seedling elms that exists on diseased field trees. It was found that elm seedlings would develop typical wilt symptoms in addition to vascular discoloration only when subjected to total darkness for 5 days, inoculated, and placed in daylight. The mechanism responsible for the production of wilt symptoms in seedlings subjected to this dark and light treatment cannot be fully explained; the high incidence of disease resulting cannot be attributed entirely to an exhaustion of the carbohydrate reserve, since there was less disease in group A seedlings which received 12 days of total darkness. Feldman, Caroselli, and Howard (6, 7), working with field trees, reported an apparent correlation between the carbohydrate reserve and the incidence of the Dutch elm disease symptoms. They found that the carbohydrate reserve was at a very low level immediately after full leaf which just preceded the first wilt symptoms. Furthermore, these investigators (8) were able to suppress wilt symptoms in field trees by spraying the foliage or directly injecting sugar solutions. It may be that the 5-day dark treatment of seedlings produces physiological conditions similar to those existing in field trees just after full leaf. Unfortunately, it was not possible to make carbohydrate determinations on the elm seedlings receiving the various dark treatments at the time of the investigation. It may be that at least two conditions are necessary in order to have a high incidence of wilt symptoms. Therefore, the hypothesis is advanced that, in addition to a possible physiological change in the host resulting from a reduction of carbohydrate or metabolite reserve, there may be a light factor responsible for this high incidence of wilting. It also appears that these two conditions must be established in the order given.

The evidence for this hypothesis is based on the following: a) Seedlings subjected to 5 days total darkness prior to inoculation and subsequently placed in light responded by 100 per cent wilt and vascular discoloration within 3 weeks. b) Seedlings receiving the same preinoculation treatment as (a) but placed in total darkness for 7 days afterward had no wilt symptoms. c) Trees receiving daylight, inoculated, and then kept in total darkness for 7 or 5 days gave 0 and 30 per cent wilt, respectively. d) No wilt symptoms were manifested in inoculated seedlings not receiving a pre-dark treatment, although these seedlings received the light factor after inoculation.

What physiological functions are set in motion or influenced by this so-called light-factor phenomenon is a question of considerable importance and one which has not yet been answered.

The method of treatment as used in group D (Table 1) has been employed successfully on over 2400 elm seedlings in chemotherapy investigations for the control of the Dutch elm disease. Data obtained from these chemotherapy tests on elm seedlings have been successfully applied to field trees.

SUMMARY

The inability, heretofore, to successfully inoculate elm seedlings with the Dutch elm disease fungus has seriously impeded progress on disease control investigations and chemotherapy studies.

Inoculation of elm seedlings by either the agar-disc or the spore-suspension method was not successful in producing a high incidence of disease. Disease in field trees was severe when either of these methods was employed.

A high incidence of disease was obtained by subjecting elm seedlings, in full leaf, to darkness for 5 days prior to inoculation, after which they were kept in 9-hr. daylight. This method of inoculation has been used successfully on over 2400 elm seedlings in dealing with chemotherapy investigations.

It has been hypothesized that at least two conditions appear to be necessary in order to produce a high degree of wilt symptoms: first, a physiological change in the host resulting from a reduction of the carbohydrate or metabolite reserve; and second, a light factor. It also appears that these two conditions must be established in the order given.

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ORGANIC FUNGICIDES IN THE CONTROL OF APPLE SCAB AND EUROPEAN RED MITE

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In the past 15 years there has been a considerable increase in the use of elemental sulfur spray and dust in Maine apple orchards, and a corresponding decrease in the use of lime sulfur. That this was a desirable change from the viewpoint of efficient apple production has been accepted generally. Also, those interested in the control of apple scab all seem to agree that some fungicide should be substituted for lime sulfur at least during part of the spray season. Until the recent introduction of the newer organic fungicides, elemental sulfur was about the only fungicide considered noninjurious enough for general use. Various copper materials were used locally by some workers, but spray injury prevented copper from being generally used on apple trees in Maine. While many of the deleterious effects of lime sulfur were alleviated by the use of elemental sulfur, it in turn has raised other problems. Lathrop and Hilborn (4), as well as others, have pointed out the increasing difficulties of European red-mite control when elemental sulfur is the principal fungicide used for the control of apple scab. There seems to be a definite correlation between the application of elemental sulfur and the build-up of European red mite in an orchard.

During the past few years the authors have sought a fungicide that would be effective in apple-scab control and at the same time would not contribute to the build-up of European red mite or would even be acaricidal. Two promising materials have been found to date—a mixture of glyoxalidines (Fruit Fungicide 341-C), and dinitro capryl phenyl crotonate (Arathane, or CR 1639).

THE 341 COMPOUNDS (MIXED GLYOXALIDINES)

Experimental work was begun with the mixed glyoxalidines in 1946 when Experimental Fungicide 341 (2-heptadecyl glyoxalidine) was used at the rate of 1 gal. of the slurry to 100 gal. of water. Application was made on row plots in the orchard described by Folsom (1), using a tractor-drawn, high-pressure spray machine, equipped with an 8-nozzle broom. The experimental material did not give satisfactory scab control, because of a hydrolysis of the active ingredient when formulated as a slurry (3).

In 1947 Experimental Fungicide 341-A (40 per cent glyoxalidines dispersed on Kaolin), at the rate of 2½ lb. in 100 gal., was used in the same row plots as in 1946 with only a fair degree of scab control. At harvest time there was 10 per cent fruit scab on the 341-A plots, only 1.6 per cent on the elemental sulfur plots, and 62 per cent on the unsprayed controls.

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Experimental Fungicide 341-B (33.3 per cent glyoxalidines dispersed on Kaolin) at the rate of 3 lb. in 100 gal., and Experimental Fungicide 341-C (54 per cent glyoxalidines, as acetates, dissolved in isopropanol) at the rate of 1 qt. in 100 gal., were both used in 1947. Replicated single-tree plots, consisting of five trees per treatment in a mature McIntosh orchard in Monmouth, Maine, were used for the experiments. The trees were sprayed with the usual spray schedule of seven applications per season. The spraying was done with a tractor-drawn, high-pressure spray machine on which a homemade spray boom was installed. The boom was slightly modified from that described as the Highmoor Multiple Nozzle Spray Boom by Hilborn et al. (2). Both of these materials gave reasonably good control of scab. At harvest time the 341-B plots averaged 2.6 per cent fruit scab, the 341-C plots 2.2 per cent, and the elemental sulfur plots 0.5 per cent, as compared with 28 per cent on the unsprayed controls.

Also in 1947 the work with 341-C was expanded, and a field plot was established in a large commercial orchard near Livermore Falls, Maine. Here a block of mature McIntosh, mostly 18 years old, was divided into two plots of some 125 trees each. During the pre-bloom period both plots received lime-sulfur applications. Beginning with the petal-fall spray, and continuing throughout the rest of the season, 341-C was applied on one plot and elemental sulfur on the other. Here the spraying was done with a high-pressure spray machine equipped with a Bean Spray Mast. The same plots were continued in 1948 and in 1949. No actual scab counts were made in this orchard, but it was noted during each of the 3 years that scab control was excellent and about equal on both plots.

In 1949 a dust was formulated using 10 per cent by weight of Experimental Fungicide 341-B in talc. A block of mature Northern Spy trees, mostly about 23 years old, located in Monmouth, Maine, was divided into two plots. Sulfur dust was used on both plots during the pre-bloom period and then continued on only one plot, the other receiving the 341-B dust. Again, no actual scab counts were made, but it was noted that scab control was excellent on both plots.

Striking differences did appear in the population levels of European red mite, when sulfur plots were compared with plots sprayed with 341-C. In the commercial orchard near Livermore Falls, where the large field plot was located, no acaricide was needed on the 341-C plot during any of the 3 years in which the test was conducted. In the adjoining plot, sprayed with elemental sulfur throughout the summer, two applications of an acaricide were necessary during each of the 3 years in order to keep red mite under even reasonably good control. The 341-B dust, used in 1949 on the block of Northern Spy trees in Monmouth, failed completely to aid in the control of European red mite. No difference could be observed in the mite population level of the 341-B plot compared with the elemental sulfur plot, and two applications of an acaricide were necessary to prevent serious injury to the

trees. Observations on the control of European red mite were of no value in either the single-tree plots or the row plots at Monmouth, because of the drift of mites from tree to tree.

The mode of action of the 341-C material in red-mite control is not known, but no direct acaricidal properties could be noted. It has been presumed that this material may influence the orchard fauna in a different respect from that of elemental sulfur, with the end result that certain predaceous species are permitted to multiply and keep European red mite under natural control.

Fruit Fungicide 341-C appears promising as a spray material for apples in Maine, especially for use during the post-bloom period. To all practical purposes it is equal to elemental sulfur as a fungicide for apple scab control, and at the same time seems to prevent the population of European red mite from reaching injurious levels.

ARATHANE (DINITRO CAPRYL PHENYL CROTONATE)

Experimental work was begun with Arathane for apple-scab control in 1948. Replicated single-tree plots, consisting of five trees per treatment, were selected in a mature McIntosh orchard in Monmouth, Maine. By means of the homemade spray boom previously described, the trees were given the usual spray schedule of seven applications per season, using Arathane at the rate of 1 lb. per 100 gal. At harvest time, scab control was about equal: the Arathane-sprayed trees had 4.6 per cent fruit scab, and the elemental-sulfur-sprayed trees had 4.2 per cent, as compared with 64 per cent for the unsprayed controls. This work was repeated in 1949 using the same equipment in the same orchard and scab control at harvest time was somewhat better in the Arathane-sprayed trees (5.6 per cent) than in those sprayed with elemental sulfur (9.2 per cent); 42.1 per cent in the unsprayed controls.

In 1948 and again in 1949 Arathane dust was used in rather large block experiments. This work is reported in some detail by Lathrop and Hilborn (4). It may be noted here that Arathane was about equal to elemental sulfur in scab control, but there was a striking difference in both years in the population levels of European red mite on the Arathane blocks and the elemental sulfur blocks. It was impractical to secure data of value on the control of European red mite when Arathane was sprayed on the single-tree plots. The drift of mites from tree to tree precluded any such data.

Arathane possesses both acaricidal and fungicidal properties. In Maine it was about equal to elemental sulfur for the control of apple scab. Arathane also proved to be efficient enough as an acaricide to keep red mite under satisfactory control when used in place of sulfur through the post-bloom period.

CONCLUSION

Two promising materials have been found in a search for a fungicide that will not only control apple scab, but also prevent the build-up of de-

structive populations of European red mite. In experimental tests Fruit Fungicide 341-C (mixed glyoxalidines) was efficient as a fungicide and apparently promoted the natural control of red mite. Arathane was efficient as both an acaricide and a fungicide.

The cost of a new material undoubtedly is a factor in determining whether it can be substituted for elemental sulfur in the scab-control program. When European red mite becomes destructive, the necessary applications of an acaricide may raise the cost of the seasonal spray program so much that a substitute for elemental sulfur may be economically used even though the substitute may be somewhat higher in price than sulfur. A material that proves effective not only in scab control, but also in the control of European red mite, should help in the effort to simplify and reduce the cost of the apple spray program.

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SURVIVAL OF RACES OF WHEAT STEM RUST IN MIXTURES[1]

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Of the more than 200 physiologic races of *Puccinia graminis tritici* Erikss. & Henn. that have been identified since 1916, approximately half occur naturally in North America. Studies of their prevalence and distribution in the United States have shown that the predominant races differ from year to year and from region to region, but very little is known regarding the factors that influence this variation in prevalence and distribution. As the distribution of wheat varieties appears to be one of the obvious factors, the present study was undertaken to determine what influence susceptible wheat varieties have on the prevalence and distribution of races of stem rust.

Results of annual surveys of physiologic races of stem rust in North Dakota from 1938 to 1943, inclusive, formed the basis for the studies (9, 10, 11, 12, 14, 15). During this period Ceres was the principal variety of bread wheat and Mindum was the principal variety of durum on which stem rust was collected in that State; therefore, most of the isolates of wheat stem rust identified during that period were obtained from these varieties. The results can be summarized as follows: 1) Race 56 was isolated from bread wheat (mostly Ceres) (3, 7) four times as often as race 17, which was half as prevalent as race 56 in the entire United States, even though Ceres appears to be equally susceptible to both races; 2) race 19 was isolated more frequently from Mindum than was race 17, although the latter was six times as prevalent as race 19 in the entire United States, and Mindum appears to be equally susceptible to both races. Thus, it appeared that certain varieties of wheat favored the survival of certain races of stem rust over other races, even though the varieties appeared equally susceptible to the different races.

The barberry-eradication campaign has reduced the number of barberry bushes in the spring-wheat area of Minnesota and the Dakotas so greatly that the principal source of inoculum is from overwintering rust in the southern part of the United States and northern Mexico (13). It has been found that the uredial stage of stem rust may overwinter normally in the general area of San Antonio, Texas, and southward, but seldom as far north as Dallas, Texas (4). In the spring the uredial stage of rust may spread

[1] This paper is a condensation of a thesis submitted by the writer to the Graduate Faculty of the University of Minnesota in 1949 in partial fulfillment of the requirements for the degree of Doctor of Philosophy. It is based on cooperative investigations between the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station. Paper No. 2532 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

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from overwintering infection centers in this area to adjoining plants, then to adjoining fields. Cyclonic winds may carry the spores northward, the effective distance depending on the moisture, temperature, and stage of development of the host in the area to which the spores are carried and on the distance the winds blow northward. Consequently, the rust may spread to the Canadian border and beyond, usually in several waves of infection (6).

The wheat stem rust in the overwintering area of northern Mexico and southern Texas at the beginning of the growing season may therefore go through many uredial generations before it is found at harvest time in North Dakota. This rust usually comprises a mixture of races and represents an ecological association of different biotypes of the same species in essentially the same environment. Since there is no evidence of direct effect of one race on another, any differential ability of the races in the mixture to survive through repeated generations is probably due to the differential effect of environment on each race in the mixture. In the studies reported here the differential effect of that part of the environment made up by the host was investigated by growing mixtures of two known races of stem rust on susceptible seedling plants of a certain wheat variety, and transferring the mixture to the same variety for several consecutive generations.

Investigations of the relative survival ability of mixtures of biotypes, races, or varieties within a species have been made. Several studies with mixtures of wheat and of barley (1, 5, 17, 18) grown for 4 to 16 years have shown that after several generations certain varieties would predominate in the mixtures, and it is pointed out that the dominance of a given variety is due not only to the effect of one variety on another but also to the response of that variety to the factors in the environment. Hoppe (2), working with equally pathogenic strains of *Diplodia zeae*, found that when mixtures of these strains were injected into corn ears only one of the strains survived, and he showed that this was the result of strong antagonism between the strains. Tapke (19) inoculated Odessa barley with a mixture of races 3 and 6 of *Ustilago hordei*, to which Odessa is susceptible. The races in 25 smutted heads were determined and race 3 was isolated from 22 heads, while only eight isolates of race 6 were obtained, indicating a better survival of race 3.

Watson (20) cultured various mixtures of five races of *Puccinia graminis tritici* on seedlings of susceptible varieties of wheat for five generations in the greenhouse. In a given mixture of races the original proportions of races changed in each generation, resulting in an increase of one race from generation to generation, whereas another race decreased. Watson suggested that the following factors might be responsible for these changes: 1) The amount and character of each race in the mixture; 2) the variety on which the mixture is cultured; and 3) the effect of temperature on the fungus. In field plots, where epidemics of stem rust were produced by inoculating susceptible border rows with a mixture of a large number of races, he found that less than half the races used in the primary inoculations were

recovered from the plots later in the season, indicating that certain races had better survival ability than others.

MATERIALS AND METHODS

A useful method for studying the ability of races of stem rust in mixtures to survive through several generations was suggested by Stakman, Popham, and Cassell (16) and diagrammed by Stakman and Loegering (9). Watson (20) utilized the method in competition studies with races of stem rust of wheat. In the studies reported here the same method was used with certain modifications. Seedling plants grown in 4-in. pots were used throughout the work, and inoculations were made by the method described by Stakman, Levine, and Loegering (8) for inoculating large numbers of pots. The cultures of races used in the experiments were obtained from field collections made away from known barberries, and the purity of the races was verified both before and after each experiment. All experiments were started in February and completed sometime in May or June, depending on the number of generations involved. Greenhouse temperatures ranged from 65° to 100° F. but were usually near 70°, and no artificial light was supplied.

Test varieties and a differential wheat variety were inoculated with a mixture of two races at a time in each experiment. The test varieties were chosen because they appeared to be equally susceptible to all races in the mixture and because certain of the races were found more commonly on one of the varieties in the field. A differential variety was chosen on which each race in the mixture produces distinctive infection types. To determine the proportion of the races on a test variety in a given generation, counts were made of the number of infections of each type on the differential variety inoculated from the particular test variety. The ratio of the two types of infection represents the ratio of the inoculum of the two races in the mixture.

The serial inoculations made in a typical experiment are diagrammed in figure 1 and were as follows: 1) Pure cultures of the two races to be studied in a mixture were established on Little Club wheat; 2) spores of each of these races were mixed, attempt being made to obtain them in equal proportion, and they were then used to inoculate another pot of Little Club wheat (generation 1); 3) the spores from this pot were used to inoculate one pot of each of the test varieties and one pot of the differential variety (generation 2); 4) each of the infected pots of the test varieties was used to inoculate three pots of the same variety and one pot of the differential variety (generation 3); 5) each of the resulting infected test varieties was used to inoculate one pot of the same variety and one pot of the differential variety (generation 4). This procedure was continued until the end of the experiment. Watson's (20) statistical study shows that this method will give a valid estimate of the ratio of the races in the mixture.

There were, however, many chances for errors in this method, because of the large number of readings and successive inoculations necessary. The

present writer found that root rot occasionally made it difficult to maintain a mixture from generation to generation and to obtain accurate counts of the infection types on the differential varieties. Occasionally there was difficulty in making accurate counts of the infection types on the differential variety because infections were so heavy that pustules coalesced. Occasional very light infections did not give an adequate number of pustules on

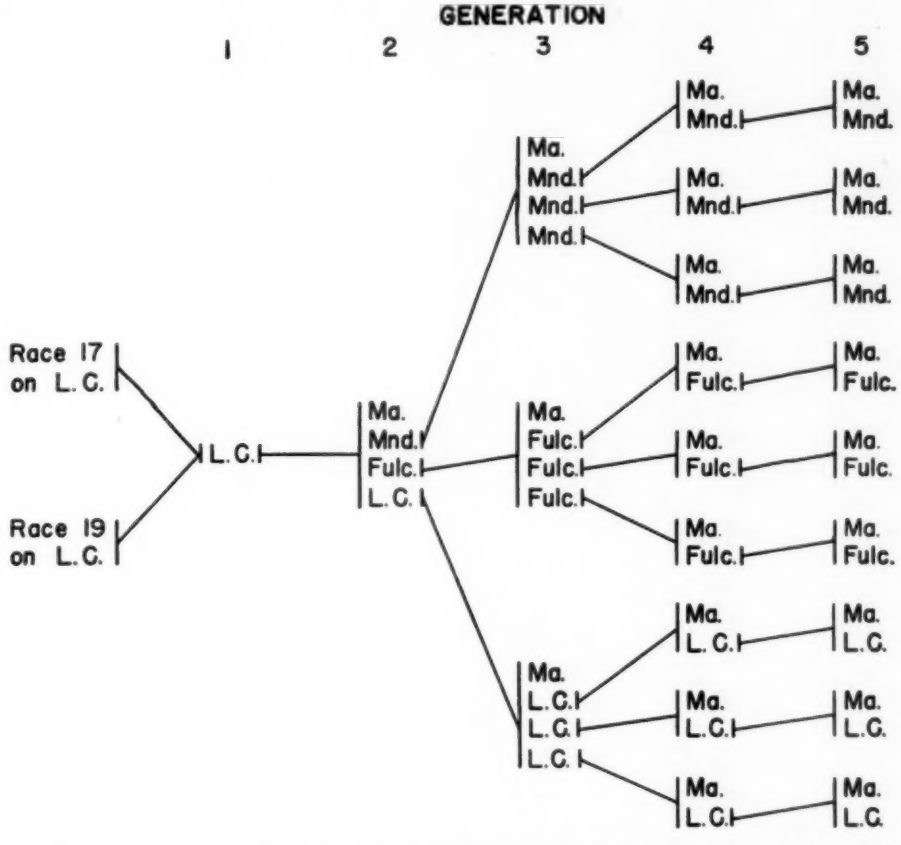

FIG. 1. Serial inoculations made in studying survival through five generations of physiologic races 17 and 19 of *Puccinia graminis tritici* in a mixture cultured on susceptible varieties of wheat. Ma.—Marquis (the differential variety); Mnd.—Mindum; Fulc.—Fulcaster; L.C.—Little Club.

which to compute a ratio. Attempt was made to reduce these sources of error to a minimum, but complete elimination was not possible.

The number of infections of each type was counted on 10 infected leaves of the differential variety. The average number of infections per leaf was approximately 40, which means that about 400 pustules were classified from each pot of the differential variety. However, because of the difficulties mentioned the number actually ranged from less than 100 in a few pots to more than 1,000 in others. In each experiment, from 58 to 67 pots of the

differential variety were required, and a total of seven experiments were made; thus approximately 200,000 pustules were classified in these studies.

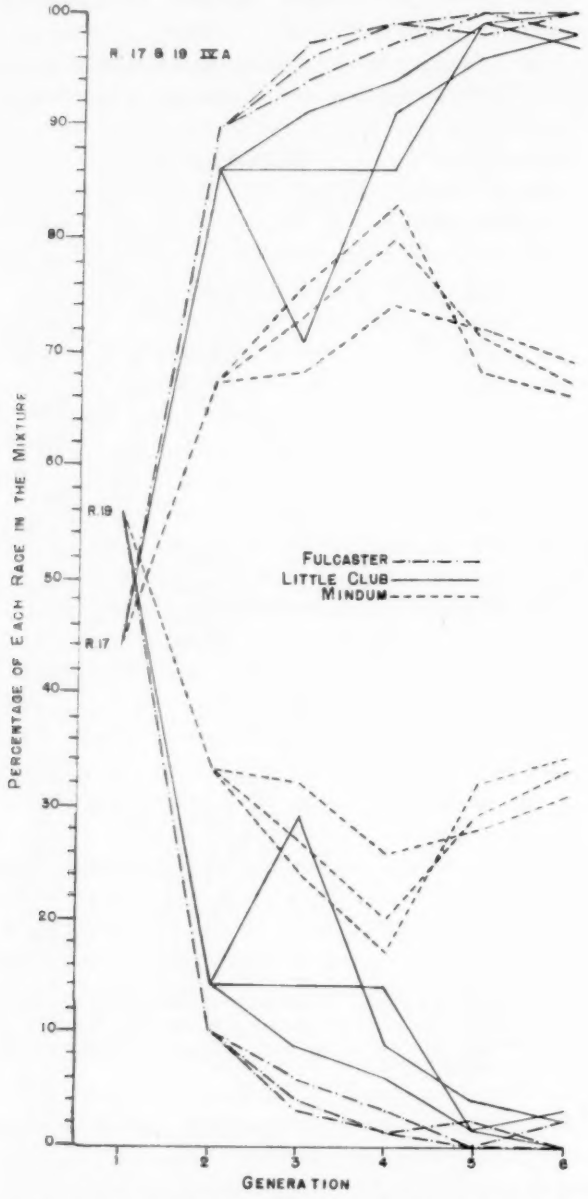

FIG. 2. Percentages of races 17 and 19 of *Puccinia graminis tritici* in a mixture cultured on three susceptible varieties of wheat for six generations, showing uniformity of the three replicates with each variety.

Despite the various sources of error, results were remarkably uniform. Since replication in each experiment began in the third generation it is

possible to compare the uniformity of results. In figure 2 are diagrammed the detailed results of an experiment with a mixture of races 17 and 19 on three test varieties. There is great uniformity in the percentage of each race on a given variety in any one generation in each of the three replicates. This same tendency was observed in all experiments; consequently, the results given in figures 3 and 4 are the averages of the three replicates on

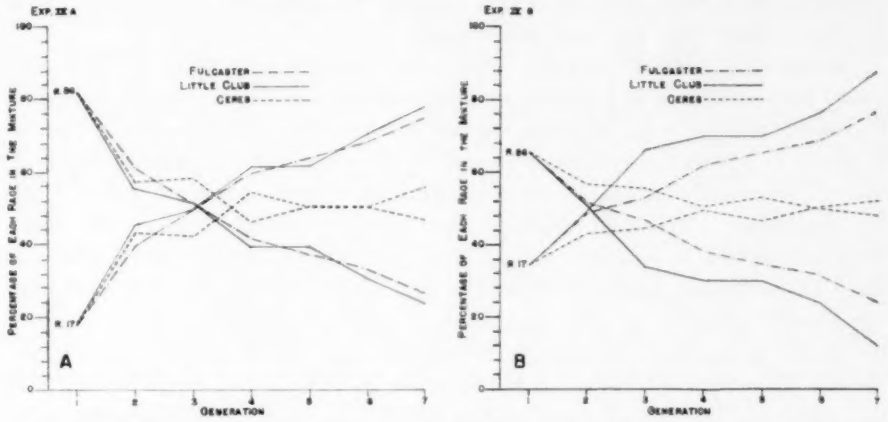

FIG. 3. A and B. Results of two tests showing percentages of races 17 and 56 of *Puccinia graminis tritici* in a mixture cultured on three varieties of wheat for seven generations.

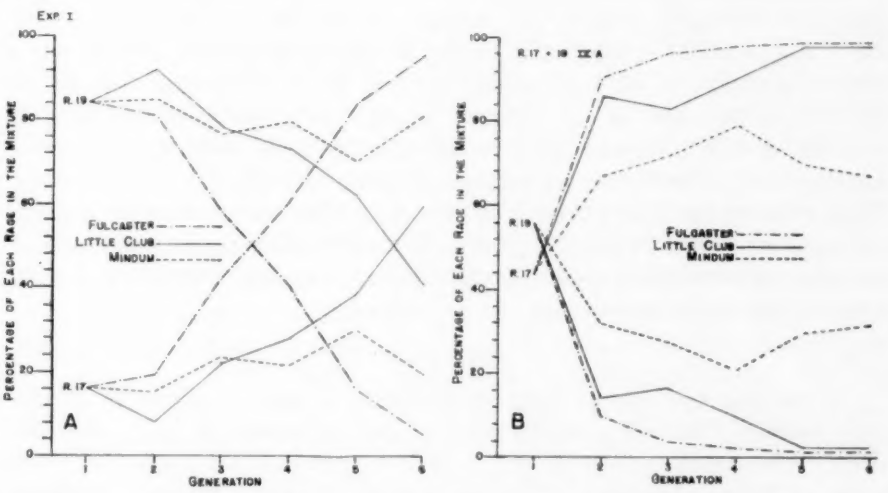

FIG. 4. A and B. Results of two tests showing percentages of races 17 and 19 of *Puccinia graminis tritici* in a mixture cultured on three varieties of wheat for six generations.

each test variety in the experiment. Thus the ratio of races in generations 1 and 2 in these figures is based on a single determination, whereas in all other generations the ratio is based on an average of three determinations.

RESULTS

Before starting the work, observations were made on the cultures of races used in these experiments to find out whether they differed in time required for sporulation and in percentage of viable spores produced. This preliminary work showed that sporulation of each race on each of the test varieties began within a few hours of each other and that germination of fresh spores of each race from each test variety was over 95 per cent in three replicated tests on 1 per cent water agar.

Mixtures of races 17 and 56.—Three experiments were made in the greenhouse with mixtures of races 17 and 56 cultured on Ceres, Little Club, and Fulcaster wheats, with Mindum durum as the differential variety. Figure 3, A and B, shows the results of two of the experiments. In all experiments race 17 gradually increased from generation to generation on Little Club and Fulcaster. The proportion of the two races remained nearly the same from generation to generation on Ceres, with a slight advantage to race 17. Race 17 increased markedly from the first to the second generation in one experiment (Fig. 3, A), but this increase may have been due to error, since the ratios in the first and second generations were based on single determinations. Thus race 17 survived better than race 56 on all three susceptible test varieties, but the survival ability differed on each of the varieties, with the least difference between the two races on Ceres.

Mixtures of races 17 and 19.—Four experiments were made in the greenhouse with a mixture of races 17 and 19 cultured in Mindum durum, Little Club, and Fulcaster wheats, with Marquis as the differential variety. Figure 4, A and B, shows the results of two of these experiments. There was a general tendency in all experiments for race 19 to be eliminated from the mixture on Fulcaster in early generations and on Little Club in later generations, and to maintain itself on Mindum or to be eliminated from the mixture much more slowly on this variety than on Little Club or Fulcaster. Thus, even though Little Club, Fulcaster, and Mindum appear to be equally susceptible to races 17 and 19, there were considerable and consistent differences in the survival of these races in mixtures on each of the hosts in these experiments in the greenhouse.

DISCUSSION

It has long been known that the distribution and prevalence of physiologic races of *Puccinia graminis tritici* may vary seasonally and geographically. Certain races have tended to predominate year after year in certain areas of North America, where there were isolation factors. However, there has also been a tendency for certain races to predominate over others in certain grain-growing areas of the Mississippi Basin, where there are no natural barriers. In some cases the pattern of distribution of races clearly is due to the relative susceptibility of the wheat varieties grown. As an example, in areas where Tenmarq—or varieties with the immunity of Kanred—is grown, races 17 and 19 would naturally be screened out.

There are, however, some unexplained patterns of distribution. In North Dakota, for example, during the period from 1938 to 1943, inclusive, a high percentage of the isolates from Ceres wheat always were of race 56, even though race 17, which appears to be equally virulent on Ceres, was very prevalent in the areas from which the initial inoculum came. In fact, the ratio of race 56 to race 17 was higher on Ceres wheat in North Dakota than the ratio in the United States as a whole. Similarly, although races 17 and 19 are about equally virulent on durums, the ratio of race 19 to race 17 was much higher on durum wheats in North Dakota than on wheats generally in the United States.

In the studies reported in this paper, the results of greenhouse experiments did not agree with observations in the field. The field observations indicated that race 56 developed better than race 17 on Ceres wheat, and race 19 developed better than race 17 on Mindum. In the greenhouse experiments with seedling plants, however, race 17 showed a slight tendency to predominate over race 56 when cultured in mixtures on Ceres, and to predominate slightly over race 19 when they were cultured together on Mindum. On the varieties Fulcaster and Little Club, race 17 consistently predominated by a considerable margin over races 19 and 56, when grown in mixture for several successive generations. Obviously, therefore, these races survived to different degrees on different varieties when grown in mixtures, even though the varieties appear to be equally susceptible. The reasons for this fact did not become apparent in the observations and experiments made by the writer.

It appears, therefore, that there are factors not clearly evident which affect the relative survival of races in mixtures. It seems probable that the concurrence of a number of relatively minor factors may bring about cumulative results when operating together for several successive generations, even though their effects might not be apparent in a single generation.

Moreover, it seems evident that virulence alone may not be sufficient to enable a race to become prevalent in nature, as this is only one of the factors that affect survival ability. Obviously, a race cannot become prevalent unless it can attack a considerable population of susceptible plants. But this alone does not guarantee its success, as it must be able to propagate abundantly under the climatic conditions to which it is subjected. It seems clear, both from observational and from experimental evidence, that combinations of relatively minor ecological factors, when operating together, affect the success or failure of physiologic races in nature.

SUMMARY

In studies of the prevalence of races of *Puccinia graminis tritici* in North Dakota from 1938 to 1943, inclusive, it was observed that on Ceres wheat the ratio of isolates of race 56 to race 17 was higher than on all grain throughout the United States, and on durum (mostly Mindum) the ratio of

isolates of race 19 to race 17 was much higher than on all wheat in the United States.

In the greenhouse, mixtures of two known races of stem rust growing on susceptible seedling plants of a certain variety were transferred to the same variety for several successive generations, and the racial components in the population were analyzed in each generation.

A mixture of races 17 and 56 was grown for seven successive uredial generations on Ceres, Fulcaster, and Little Club wheats, all of which appeared to be equally susceptible to the two races. On Fulcaster and Little Club race 17 became predominant by a wide margin, while on Ceres it predominated only slightly.

In the same manner, a mixture of races 17 and 19 was grown for six successive uredial generations on Mindum durum, Fulcaster, and Little Club wheats, all of which appeared to be equally susceptible to the two races. On Fulcaster and Little Club race 17 predominated after a very few generations, while on Mindum race 17 predominated only slightly after six generations.

Evidently the relative ability of races to multiply in mixtures is not due alone to observable differences in virulence. It seems likely that relatively minor ecological factors may combine to affect the differential development and survival of races in mixtures that occur commonly under natural conditions.

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ANATOMICAL SYMPTOMS USEFUL IN DIAGNOSIS OF LILY ROSETTE[1]

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Lily rosette was named "yellow flat" when first comprehensively investigated in Bermuda by Ogilvie (8), who established it as a virus disease transmissible by *Aphis gossypii* Glover. This disease has been found in American plantings of *Lilium longiflorum* Thunberg, twice in Florida and once in Texas. These records were confirmed by aphid transmission tests, and one of the Florida cultures is still maintained at the Plant Industry Station, Beltsville, Maryland. An additional collection from Louisiana by A. G. Plakidas in March 1949 has been confirmed by the presence of anatomical characteristics herein described.

Yellowing, rosetting, and downward curling of leaves as commonly illustrated (2, 8) are distinctive, particularly for current-season infections when normal basal leaves are contrasted with rosetted upper leaves. Diagnosis is not difficult in field plantings when various stages of rosetting are present and normal plants are available for comparison. Yellowing is less conspicuously developed in greenhouse pot culture, and the curling of the leaves is irregular and less distinctive in second-season expression. Root diseases such as Fusarium rot, injuries to roots or to the stem, certain forms of frost damage, and as shown by Smith and Brierley (9), the specific injury caused by the feeding of *Myzus convolvuli* (Kalt.) may be mistaken for rosette symptoms. Single plants submitted for examination are often impossible to diagnose by appearance alone without further confirming evidence. Subinoculations by means of the specific vector, *Aphis gossypii*, are decisive since this virus is carried with high efficiency; but such tests are feasible only when nonviruliferous colonies of the vector and test plant are available. A simpler and faster method of confirming diagnosis is needed. For these reasons rosette plants were examined for anatomical and cytological changes. Evidence is presented that distinctive anatomical symptoms are regularly present and useful for diagnosing lily rosette.

The only previous record of a histological or cytological study of yellow flat is a paper by Dufrenoy (3). Critical reading of this paper shows he was investigating a lily mosaic disease, not yellow flat. His findings are irrelevant to the present discussion.

[1] Cooperative project between the Oregon Agricultural Experiment Station and the Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture; published with the approval of the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 614. Contribution of the Department of Botany and Plant Pathology.

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MATERIALS AND METHODS

Pure cultures of the lily rosette virus in Easter lily seedlings were chosen for study. The virus was freed from other lily viruses commonly accompanying it in commercial stocks by techniques published previously (1). Nonpersistent viruses of the lily-mottle and cucumber mosaic groups were eliminated by feeding the vector on a nonsusceptible plant for a few days. In serial transfers by means of the melon aphid, the persistent lily symptomless virus tends to be lost and the lily rosette virus, also persistent, is retained because of higher efficiency of transfer. Seedlings with current-season, and also seedlings with second-season, symptoms of rosette were studied.

Freehand sections of living leaves were examined in 0.05 per cent trypan blue made up in physiological salt solution (7). This dye selectively stains the significant necrosis and is recommended for rapid diagnosis of the disease. Material fixed in aceto-formalin was processed in paraffin by the dioxan (6) and tertiary butyl alcohol methods, then stained, or mounted for microchemical tests.

PATHOLOGICAL ANATOMY

The anatomical symptoms of this disease are usually confined to the vascular elements and the supporting bundle sheaths (Fig. 1, B). The conspicuous symptoms, observable in cross-sectioned bundles, are necroses in phloem and adjoining sheath tissues. The symptoms do not classify clearly into any one of the groups of phloem necrosis tentatively suggested by Esau (5). The necroses of lily rosette are more generalized than her "a" group, for which potato leaf roll is the type. In several ways the symptoms observed closely resembled those produced by curly-top virus in the vascular elements of tobacco and beet leaves, as ably described by Esau (4). There were, however, three significant differences: 1) The necrotic areas were more restricted and less conspicuous than those of curly top. 2) In the lily there was no evidence of necrotic products extruded into exudate channels extending through the leaf to the epidermis. 3) There was no conclusive evidence of hypertrophy or hyperplasia in any of the affected lily tissues.

The appearance of individual necroses varies according to the kind and age of cell that degenerated. From sections of leaves of typically diseased plants we conclude that the order of frequency for degeneration of cells is 1, companion cells; 2, sheath cells and adjacent sieve tubes; and 3, phloem and xylem parenchyma. Very rarely do lesions extend beyond the sheath cells into the supporting parenchyma. For clarity, each type of these lesions is discussed separately and the specificity of the degenerative process for each is emphasized for possible use in diagnosis.

Typical companion cells were seldom observed in bundles where necroses were discernible. They were obliterated to deeply staining points. Application of this observation requires careful comparison with control prepara-

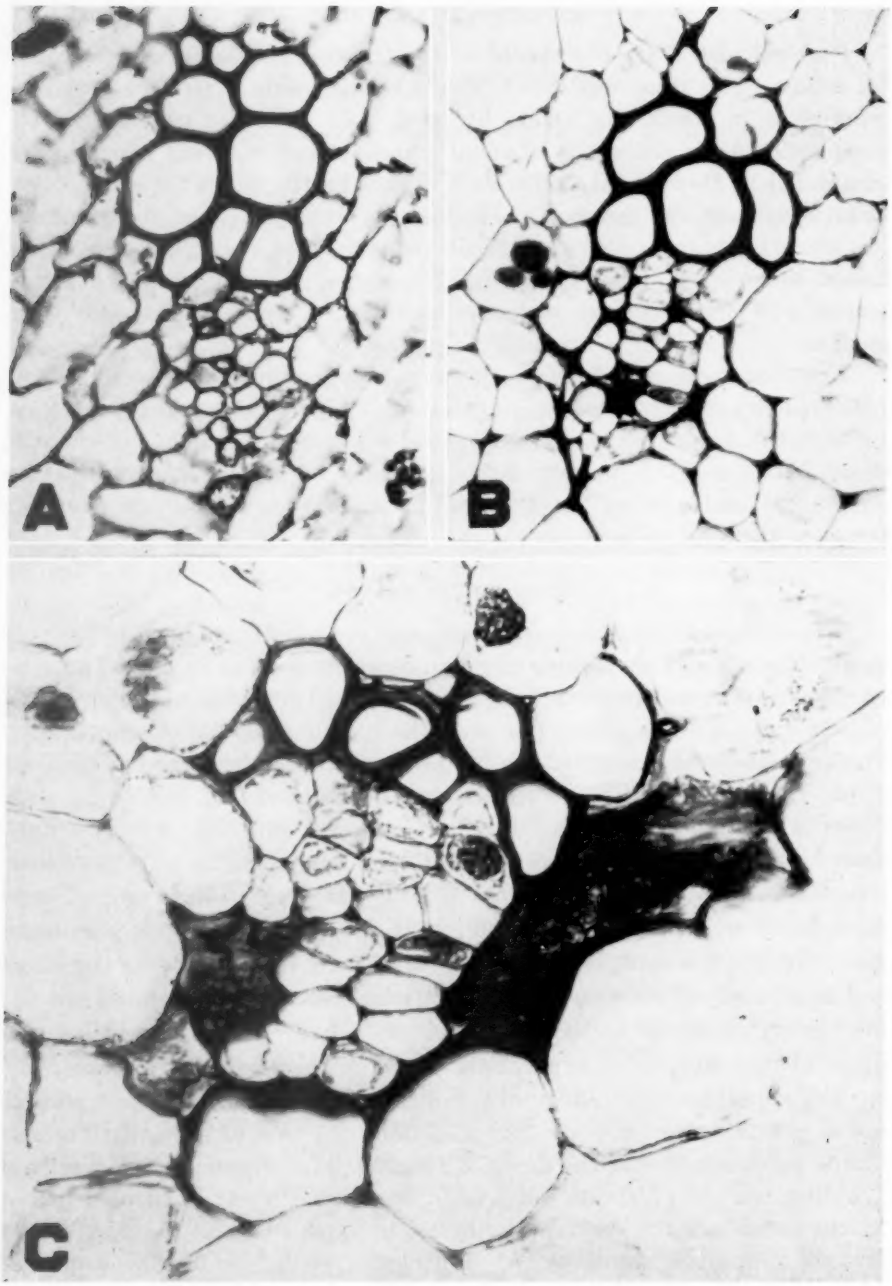

FIG. 1. Cross sections of normal and rosette-diseased leaves of *Lilium longiflorum*. A. Vascular bundle in normal leaf. (500×.) B. Necroses within phloem tissue and in areas between phloem and sheath cells. All companion cells have been obliterated. Note lobes of enlarged nucleus in parenchyma cell to the left of bundle. (500×.) C. Advanced necrosis in phloem and bundle sheath regions. Note abundant extruded materials. (1000×.)

tions and is of little practical use for diagnosis. However, the consistent absence of these cells in bundles that are otherwise normal indicates they are the first cells to show the effects of the virus.

Large xylem parenchyma cells when seen in longitudinal sections show clearly progressive stages of degeneration. A series studied with the Feulgen technique and with several stains indicates that their degeneration begins with the accumulation of basophilic masses of spheres 0.5 to 1.0 μ in diameter around the nuclei. When this largely proteinaceous material has filled about one-third of the cell, the nucleus starts to disintegrate. At this stage the Feulgen test is needed to distinguish the material added or made visible by the virus from the initial nuclear material. This is followed by collapse of the cell and by the accumulation of lipoid products sometimes present as fat crystals. These changes are somewhat specific for this virus but are impractical for diagnostic use. However, the presence in cross sections of degenerated cells adjoining the differentiated xylem is of real diagnostic value.

The necroses in the sheath cells and adjoining phloem elements (Fig. 1, C) are conspicuous and highly significant for diagnosis. Freehand sections of living leaves mounted in trypan blue show these affected cells as intensely stained bluish-black masses. This necrosis has not been found to accompany any other virus disease of lily. The components of this necrosis proved very complicated when analyzed in paraffin sections with dyes and microchemical tests.

The lesions in the sheath-phloem region stained differentially with methylene blue diluted 1/25,000, with methyl green diluted 1/15,000, and with chlorozinc-iodide. Here the degeneration process forms deeply staining material within affected cells and lamellated cell-wall-like structures around and between the affected cells. The lamellated material is not birefringent and stains like lignified walls with the usual botanical stains. Microchemical tests for lignin indicated that no lignin was present. The innermost lamella is frequently cutin and tests to the same end point for cutin with Scharlach R as the cutin of the epidermis in the same sections. We suggest that these lamellated wall-like structures are formed by contiguous uninjured cells as a protection against the necrobiotic processes which attend cell degeneration. This thickened wall phenomenon is easily seen in paraffin sections and can be seen in good freehand sections, especially if the latter are immersed in very dilute methylene blue. This lamellated material and accompanying amorphous products are characteristic of rosette and are of specific diagnostic value.

While we were unable to prove hyperplasia or hypertrophy as part of the disease picture, there may be enlargement and excessive lobing of nuclei in sheath areas. All of the test plants and comparable controls were individual seedlings. Nuclei estimated to be from four to eight times the usual size were frequent in cells of the diseased seedlings near the necroses. Some of these were so abnormal that we could not accept them as nuclei

until the Feulgen technique proved their identity. The validity of enlarged nuclei as a symptom cannot be tested until diseased and healthy plants belonging to the same clon are available for study.

Symptoms similar to the above occur in the vascular elements of the stems of affected plants. The progression of symptoms and their distribution throughout the plant have not yet been studied.

THE VALUE OF VASCULAR NECROSIS IN LEAVES FOR DIAGNOSIS

Since this disease is rare in the United States the material available has been limited. Typical necroses were found in six plants infected the second year of infection and in six plants infected during the current season. All plants proved to be infected by aphid transfer had the histological symptoms described above.

Counts from sections of individual leaves were made to determine the number of bundles having symptoms versus the total number of bundles. The following distribution was recorded for five similar leaves from five different plants: 9/22; 10/19; 13/17; 15/16; 26/27. These counts were made from sections of living leaves mounted in trypan blue. From paraffin sections we have concluded that from 60 to 95 per cent of the bundles of a section 6 μ thick may be expected to show symptoms. All tests indicate that necrosis in vascular bundles is a consistent symptom of the disease.

These anatomical symptoms are certainly more specific than the external symptoms. Moreover, such symptoms are not produced by the other virus diseases of *Lilium longiflorum*, namely, the fleck complex and the mottle or mosaic disease. In the case of the fleck complex, the cell degeneration processes are largely confined to the chlorenchyma, and typical necrosis, as that term is generally used, may not ensue. The vascular elements within fleck areas remain as normal-appearing islands of cells surrounded by the partly coagulated chlorenchyma cells. Cross sections of bundles where mottle virus is present have no anatomical or cytological evidence of the virus. The necroses described in the vascular areas of rosette-diseased lilies seem to be of specific use for diagnosis of the disease.

These necroses are not readily discernible in freshly cut freehand sections of living leaves mounted in water. However, if the sections are mounted in 0.05 per cent trypan blue made up in 0.85 per cent salt solution, they are easily seen, since the necroses stain quickly to a bluish-black end point. This dye is a general protein stain. The phloem, having a relatively high protein content, stains darker than other tissue and appears in sharp contrast to xylem, which is unstained. Before attempting to use this test for the necroses of rosette infection one should apply the stain to sections of appropriate controls to observe the appearance of normal phloem selectively stained with the dye. The selective staining of necroses ensues within 10 to 20 sec. after mounting in the dye. This test for rosette disease should require about 5 min. for completion.

SUMMARY

Necrosis of the vascular elements occurs regularly in leaves of *Lilium longiflorum* infected with rosette virus. The necroses are usually in the phloem elements and sheath cells. Occasionally they occur in cells adjacent to the sheath cells and in xylem parenchyma. Such necroses do not occur in the foliage of lilies infected with other virus diseases. They are easily demonstrated in freehand sections of living leaves mounted in 0.05 per cent trypan blue. Since the necroses are regularly present, testing with trypan blue should prove a rapid method of diagnosing the disease.

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FUSARIUM FOOT ROT OF SWEET-POTATO SPROUTS[1]

T. T. McCLURE[2]

(Accepted for publication September 6, 1950)

During the course of an investigation of the Fusarium wilt of sweet potatoes (*Ipomoea batatas* Poir.) (6), some isolates were obtained of *Fusarium solani* (Mart.) App. & Wr. emend. Snyd. & Hans. which were able to attack sweet-potato sprouts. The pathogenicity, cultural characteristics, and the part played in the Fusarium wilt of sweet potatoes by these isolates comprises the body of this paper.

Fusarium solani, both in its conidial and ascigerous (*Hypomyces solani* Rke. & Berth. emend. Snyd. & Hans.) stages, has come into question at various times as a possible pathogen of sweet potatoes. Under the name *Nectria ipomoeae* Hals., it was considered the causal organism of the Fusarium wilt disease (2, p. 281), but Harter and Field (3) demonstrated by inoculation tests that it did not cause that disease. They did note, however, a black foot rot on some of their inoculated plants, which they attributed to improper greenhouse conditions. *N. ipomoeae* was found to be incapable of causing a rot of stored sweet potatoes (5), although it could frequently be isolated from decayed potatoes and from rotted portions of plants affected with the wilt (4). The ascigerous stage of *N. ipomoeae* was claimed to be readily derived from pure cultures started from conidia (4), but Dimock (1), using a single-spore transfer technique, found that his fungus, which he called *Hypomyces ipomoeae* (Hals.) Wr., was composed of two cross-fertile strains, each of which was hermaphroditic and self-sterile.

MATERIALS AND METHODS

Seed sweet-potato roots of the Porto Rico Red variety were obtained after storage from growers in the San Joaquin Valley of California. A large number of these roots had brown, shrivelled ends which were brown and dry internally. When isolations from 33 such end lesions were made on acidulated potato-dextrose agar, 15 of the isolates were identified as *Fusarium oxysporum*, 10 as *F. oxysporum* f. *batatas*, seven as *F. solani*, and one as *F. roseum*. The isolates were maintained by single-spore transfers to culture tubes of potato-dextrose agar. For inoculation purposes, approximately 400 ml. of a spore-suspension inoculum could be made from a 2-weeks-old culture.

Healthy sprouts were produced from other seed sweet-potato roots which were bedded in sand in an electrically heated hotbed. Sprout cuttings were

[1] A revision of a section of a thesis submitted to the Graduate Division of the University of California at Berkeley in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to acknowledge his indebtedness to Dr. William C. Snyder and other members of the Division of Plant Pathology for counsel and advice in this work.

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inoculated by dipping the fresh basal wound in a spore suspension. Some healthy cuttings were grown in steamed sand until the basal wounds were completely healed, after which they were inoculated by dipping the roots and stem bases in a spore suspension. Great care was taken that no roots were broken or damaged during transplanting. At the end of an experiment, visual examination to determine infection was supplemented by isolations from plant parts after surface disinfection with mercuric chloride (1:1000) in 10 per cent ethyl alcohol.

Inoculation of fresh cuttings.—When the seven cultures of *F. solani* were tested for pathogenicity to freshly cut sweet-potato sprouts, four of them were nonpathogenic and three caused a limited black rot of the stem base (Table 1). Infected plants had pale-green leaves and were retarded some-

TABLE 1.—*Results of inoculation of fresh cuttings of sweet potato with seven cultures of Fusarium solani obtained from rotted seed sweet potatoes*

Culture number of inoculum	Number of plants in test	Number of plants infected (foot rot)
Control	74	0
22	59	56
80	15	15
92	15	12
18	15	0
30	19	0
73	15	0
90	35	0

what in their growth for approximately 2 weeks, after which they grew normally. The foot rot spread from the basal wound up the stem but seldom extended beyond the basal node (Fig. 1, A and B). Both the pith and the cortex were invaded by the fungus, and the xylem was discolored for approximately $\frac{1}{2}$ in. in advance of the rot. The stem was frequently found to be split open at the base, but this splitting is not considered diagnostic of the disease since a similar splitting was caused by *F. oxysporum* f. *batatas* and by physiological disturbances.

Inoculation of healed cuttings.—Sweet-potato sprout cuttings whose basal wounds were allowed to heal prior to inoculation with a pathogenic culture of *F. solani* (Table 2) remained free of any rot for the duration of the experiments, the longest of which lasted 8 weeks (Fig. 1, E). When the callus of such healed cuttings was cut off at the time of inoculation, a limited black rot occurred in the region of the fresh wound. Uninjured root tips were not attacked. Cut roots were rotted for less than 1 cm. from the wound (Fig. 1, F). Sometimes the large eruption wounds produced on the stem by the emergence of adventitious roots developed a limited and superficial black rot. Thus the causal organism is a wound parasite which normally does not produce extensive damage.

This disease is tentatively termed the Fusarium foot rot of sweet pota-

toes to distinguish it from the foot rots caused by *Plenodomus destruens* Harter and *Macrosporium solani* E. & M. A foot rot produced by the wilt pathogen (*F. oxysporum* f. *batatas* (Wr.) Snyd. & Hans.) is accompanied

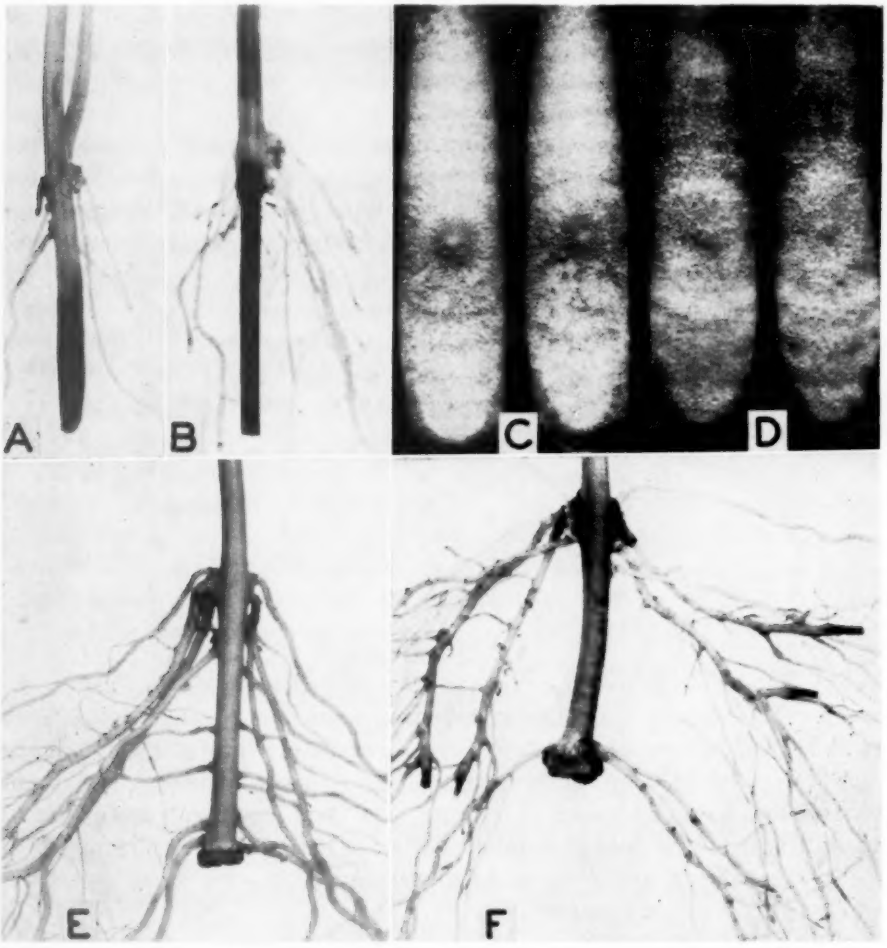

FIG. 1. A. Base of sweet-potato sprout, 1 week after inoculation of fresh basal wound with the foot-rot Fusarium. B. The same, 2 weeks after inoculation, showing extent to which infection progresses. C. Cultures of foot-rot Fusarium (*F. solani* f. *batatas*) 1 month old on potato-dextrose agar. D. Cultures of nonpathogenic *F. solani* 1 month old on potato-dextrose agar. Note similarity between pathogenic and nonpathogenic cultures, both originally isolated from sweet potato. E. Base of sweet-potato plant 2 weeks after inoculation with the foot-rot Fusarium. Basal wound was healed (note callus) prior to inoculation and no infection resulted. F. A similar base, but roots were severed at time of inoculation and have a limited infection.

by extensive vascular discoloration, by which it may be distinguished from this disease.

Description of pathogen.—The pathogenic cultures were very similar to each other, but not identical, indicating the presence of different clones.

TABLE 2.—*Results of inoculation of healed and wounded sweet-potato cuttings with Fusarium solani f. batatas*

Treatment of healed cuttings	Inoculum	Duration of test in weeks	Number of plants tested	Number of plants infected
Uninjured	None	2	10	0
Do	do	4	10	0
Do	do	8	5	0
Do	*F. solani* f. *batatas*[a]	2	15	0
Do	do	4	15	0
Do	do	8	10	0
Callus severed	None	2	10	0
Do	*F. solani* f. *batatas*[a]	2	15	15[b]
Roots severed	None	2	10	0
Do	*F. solani* f. *batatas*[a]	2	10	10[c]

[a] Culture number 22 used. [b] Basal wound rotted. [c] Severed roots rotted.

Their cultural characteristics (Fig. 1, C) on potato-dextrose agar are as follows: aerial mycelium abundant, white, and showing zonate growth; sporodochial masses buff colored and frequently confluent; 5-septate macroconidia average $54.4\mu \times 5.4\mu$. Pure cultures derived from single-spore transfers never developed perithecia, but when flooded with conidia from some of the other pathogenic isolates or from a culture of Dimock's *Hypomyces ipomoeae*, perithecia and ascospores were produced.

Two of the nonpathogenic cultures had green spore masses, and the other two were similar to the pathogenic cultures but had less aerial mycelium (Fig. 1, D). The lack of absolute cultural differences between pathogenic and nonpathogenic cultures indicates the need for pathogenicity tests for the identification of the pathogenic clones.

It is proposed that those clones of *F. solani* which are pathogenic to and cause foot rot and root rot of sweet potato be named *F. solani* f. **batatas**, n.f., in accordance with the nomenclature proposed for this species by Snyder and Hansen (8).

Effect of Fusarium foot rot on Fusarium wilt of sweet potatoes.—Since neither the Fusarium foot rot nor the Fusarium wilt pathogens are able to infect healed and subsequently uninjured plants (6), the question arose whether they might not be more virulent when both were present. Healed, uninjured plants inoculated with a mixed spore suspension of both pathogens remained healthy (Table 3). If by combining the pathogens any increase in virulence was obtained, it was not enough to result in the infection of unwounded plants.

It was then postulated that the foot-rot Fusarium, by means of its rotting action, might keep the basal wounds vulnerable to attack by the wilt Fusarium. Freshly cut sweet-potato sprouts were dipped in a spore suspension of the foot-rot Fusarium and then after various intervals in a spore suspension of the wilt Fusarium, and grown in steamed soil for 3 or 4 weeks. Contrary to expectations the results (Table 3) indicate that infection by the

TABLE 3.—*Prevention of infection of sweet-potato sprouts with the wilt Fusarium by prior infection with the foot-rot Fusarium; results of double inoculation of both freshly cut and healed sprouts, with various intervals between inoculations*

Condition of sprouts, and interval between inoculations	Number of plants tested	Number of infected plants	
		Wilt	Foot rot
Freshly cut			
5 days	20	0	20
2 days	10	0	10
1 day	15	0	15
5 min.	40	4	40
None[a]	20	18	20
Healed			
None[a]	20	0	0

[a] Single inoculation, inoculum being mixture of spores of both pathogens.

foot-rot Fusarium protected sprouts from subsequent infection by the wilt Fusarium. Such protection was 100 per cent effective when the interval between inoculations was 24 hr. or longer, and 90 per cent effective when only a 5-min. interval between inoculations was tried. The protected plants had the typical limited rot of the Fusarium foot-rot disease, but did not develop the extensive vascular discoloration typical of infection by the wilt Fusarium, nor could that pathogen be isolated from the stele above the foot-rot lesion.

When freshly cut sprouts were inoculated with a mixture of spores of the foot-rot Fusarium and of the wilt Fusarium, no protection was obtained against the wilt disease. An experiment, results of which are not included in table 3, indicated that no protection resulted when freshly cut sprouts were inoculated, first with a nonpathogenic *Fusarium solani* and then, after 24 hr., with the wilt Fusarium. Of 10 sprouts so inoculated, none were free of the wilt disease when examined 2 weeks later, and nine were obviously dying.

Both the foot-rot Fusarium and the wilt Fusarium were grown in the same Petri dish on potato-dextrose agar, but no antibiotic effects were noted between the two colonies. The mechanism of protection of sweet-potato sprouts by prior infection with the foot-rot Fusarium may lie in the xylem of the sprouts. As the foot-rot Fusarium causes but a limited rot, it may be that the tissues of an infected sprout develop a delimiting barrier which, once formed, is effective against the wilt Fusarium. Histological examinations of foot-rot-infected sprouts revealed the presence of numerous tyloses in the vessels in advance of the fungus. Tyloses are also commonly found in the vessels of plants infected with the wilt Fusarium, and the method by which that pathogen by-passes tylosis-clogged vessels by growing into unclogged vessels is described in a previous paper (7). As the sprouts infected with the foot-rot Fusarium do not become infected when subsequently inoculated with the wilt Fusarium, it may be that the foot-rot pathogen stimulates the sprout to produce tyloses in all of its vessels, thus leaving no

unclogged vessel by which the wilt pathogen could by-pass tylosis-clogged vessels. In this manner the protection of sprouts from a virulent pathogen by prior infection with a mild pathogen may be explained.

SUMMARY

Certain single-spore cultures of *Fusarium solani*, isolated from rotted sweet potatoes, were found to be mildly pathogenic to sweet-potato sprouts, causing a limited black foot rot. The infection did not progress above the basal node, nor were roots rotted for more than 1 cm. The pathogen was found to be a wound parasite incapable of attacking uninjured stems or roots. Freshly cut sprouts inoculated with this foot-rot Fusarium were protected from subsequent infection by the sweet-potato wilt fungus, *Fusarium oxysporum* f. *batatas*. This protection occurred when as short a time as 1 day or less elapsed between inoculations. Tyloses were abundant in the xylem in advance of the foot-rot pathogen.

Other isolates of *Fusarium solani*, culturally similar to the foot-rot Fusarium, were nonpathogenic to freshly cut sprouts and incapable of protecting sprouts from subsequent infection with the wilt Fusarium.

Pathogenesis for sweet potato in *Fusarium solani* has been denoted by creation of a new form, *F. solani* f. *batatas*, and the disease produced has been named the Fusarium foot rot of sweet potatoes.

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VIRUS PARTICLES IN VARIOUS PLANT SPECIES AND TISSUES[1]

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The association of virus particles, as seen under the electron microscope, with certain virus diseases of plants and animals has stimulated research and discussion relating to their bearing on the nature of viruses. The present status of the problem may perhaps be best illustrated by Sigurgeirsson and Stanley's (12) statement in 1947: "In view of the large amount of experimental data which indicates that tobacco-mosaic virus activity is directly associated with particles 15 by 280 mμ, it would appear desirable to continue to accept the 15 by 280 mμ particles as tobacco-mosaic virus until such a time as virus activity is proved experimentally to be associated with a smaller particle."

In the present study the potential association of rodlike particles with a variety of plant species, organs, and distinct virus diseases has been examined. The investigation has been greatly aided by the use of the water-pressure method of securing virus from plant tissues for purposes of electron microscopy. Two preliminary notes from this laboratory (8, 9) briefly describe the method and its results.

METHOD AND MATERIALS

A method of rapidly applying the common water-line pressure to plant tissue (7) in such a way as to quickly and conveniently supply material for electron microscopy is shown in figure 1. Lower pressures and various modifications of this equipment may be used; it has been shown, for example, that even guttation drops from tomatoes infected with tobacco mosaic contain virus (6). Some advantages result from the use of water pressure as high as 100 lb. with certain plants such as the cucumber, which does not quickly yield exudates from cut veins even under high pressure.

The equipment used to confine the water pressure is basically similar to that used in sealing valve stems. Rubber stoppers of the proper diameter are prepared with different sized bores before slitting on one side in such a way that a close-fitting orifice may be selected for different sized plant tissues. The stopper and the stem or petiole of the plant tissue are then inserted in the orifice of the water-pressure chamber, and a lock nut provided with a split metal washer holds the stopper firmly. A water-tight joint around the confined portion of the tissue normally may be prepared in less than a minute. Absorbent cotton or modeling clay may be required inside of the stopper to check leaks. When the water pressure is turned on, crystal-clear droplets from hydathodes or cut ends of veins appear almost at once,

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but some plant tissues may require several minutes for sufficient exudation to occur. The exudate may be transferred to the screen with medicine

FIG. 1. Tomato leaf under water pressure, showing droplets used for preparation of electron-microscope screens. Leaflets cut for collection of vascular contents.

droppers or small pipettes, but we preferred to place the screen on the glass slide in direct contact with the droplet. We also transferred similar drop-

lets to a suitable host plant to verify the presence, or determine the concentration, of the virus in the droplets.

In the earlier trials collodion films were used on the electron-microscope screens and gold used for shadow-casting. Considerable trouble was experienced with the breaking of the collodion film in the microscope, especially with the exudates of certain plant species. The use of "formvar" film and uranium for shadow-casting greatly improved the technique for all types of material examined. The principle of shadow-casting is illustrated in figure 2, A.

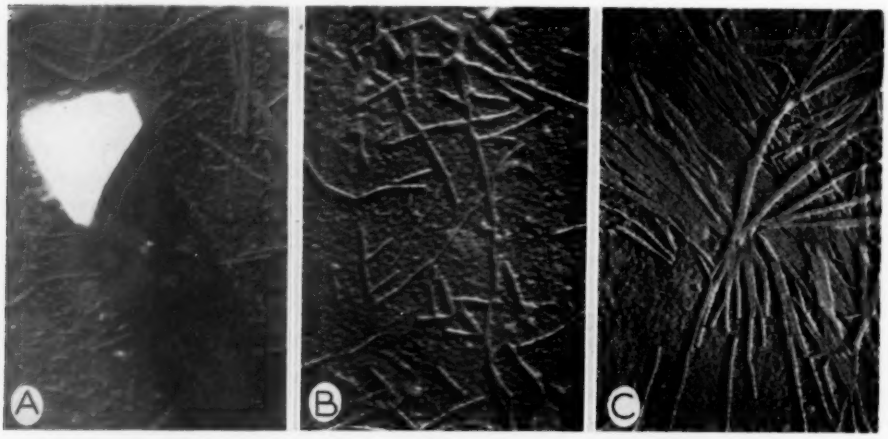

FIG. 2. A. Tobacco-mosaic virus particles from tobacco. Note variable particle length. The shadow thrown by the large piece of debris illustrates angle of shadow-casting used for electron microscopy. (×13,000.) B. Tobacco-mosaic particles from exudate of *Physalis pubescens* showing the common lack of good uniformity of particle size. (×24,000.) C. Tobacco-mosaic particles from *Solanum nigrum* illustrating frequent tendency to aggregate into crystalline plates. (×19,000.)

The viruses studied were obtained mostly from plants grown in the greenhouse, where repeated testing and verification of the virus itself and its freedom from contamination were feasible. Simultaneous investigations on strain variations within ordinary tobacco mosaic provided some additional material for observation. These strains were also being grown on different host species, which permitted comparison of virus particles from several hosts. In the instance of tobacco mosaic especially, opportunity for checking on the presence and the concentration of the virus in the vascular contents was afforded by inoculation to the sensitive hybrid (*Nicotiana tabacum* × *N. glutinosa*) local-lesion host. With some other viruses such "control" inoculation was not made, and where particles foreign to the normal vascular contents were not found under the electron microscope the results are not regarded as proof of their absence. It is our opinion, however, that low concentrations of virus particles are as likely to be detected in the vascular contents as in preparations from other tissues.

Some of the viruses were from material collected out-of-doors. In most of these instances the symptoms were typical of the disease indicated, and

it was known that the host was not subject to infection with the tobacco-mosaic virus. In other instances the absence of other common viruses could be verified by inoculation to the proper host. As a rule the host tissues chosen were young leaves with relatively new symptoms.

TOBACCO-MOSAIC VIRUS PARTICLES

Since most of the prior investigations relating to the particulate structure of viruses have been performed with the ordinary tobacco-mosaic virus, the greatest interest centers around these rodlike particles which many different workers have observed under the electron microscope. The virus for such studies has usually been prepared from relatively large masses of plant tissues subjected to a variety of treatments for purification purposes. Such treatments may naturally result in considerable disruption or aggregation (10) of the particles.

By the water-pressure method, rodlike particles can be demonstrated in all parts of systemically invaded host plants. The particles have been found in large numbers in vascular contents collected from bud leaves of tobacco 36 hr. after inoculation of the lower leaves (Fig. 5, B). In other plants, such as bitter nightshade, the progress of the virus may be very slow and uncertain as shown both by test inoculation and electron microscopy. Single droplets of exudate may be collected from small portions of almost any desired organ or area of the plant, even the flower parts and seed pods. It is believed that virus particles obtained from the ends of cut leaf veins are largely formed in phloem or xylem tissues and are not transferred or forced from other tissues into the vascular system (5). It seems apparent that the pressure involved at the point of collection of the virus is very low and could hardly influence particle size and shape or disrupt or aggregate particles, as may occur during purification by freezing, grinding, precipitation, and centrifugation. Furthermore, the droplets were dried on the films within a few minutes after they were taken from the plant. Nevertheless, we know little about the effect of drying, shadow-casting, and radiation on the particles before or during exposure under the electron microscope. While they may form parallel aggregations (Fig. 3, B) in drying on the screen, it seems very unlikely that single rods would unite end to end in such a way as to form single long threads (Fig. 2, B). Altogether it seems likely that the water-pressure method of study permits a highly satisfactory comparison of virus particles obtained from different organs of the same plant and from different host plants infected with the same virus as well as the particles from distinct viruses.

Because the tobacco-mosaic virus will systemically infect a number of other solanaceous host species as well as some species outside this family, the vascular contents of tobacco-mosaic-infected leaves from several of these other species were compared under the electron microscope. Rodlike particles were found in the vascular contents of the following solanaceous species: *Nicotiana glauca* Grah., *N. paniculata* L., *N. rustica* L., *N. sylvestris* S. & P.,

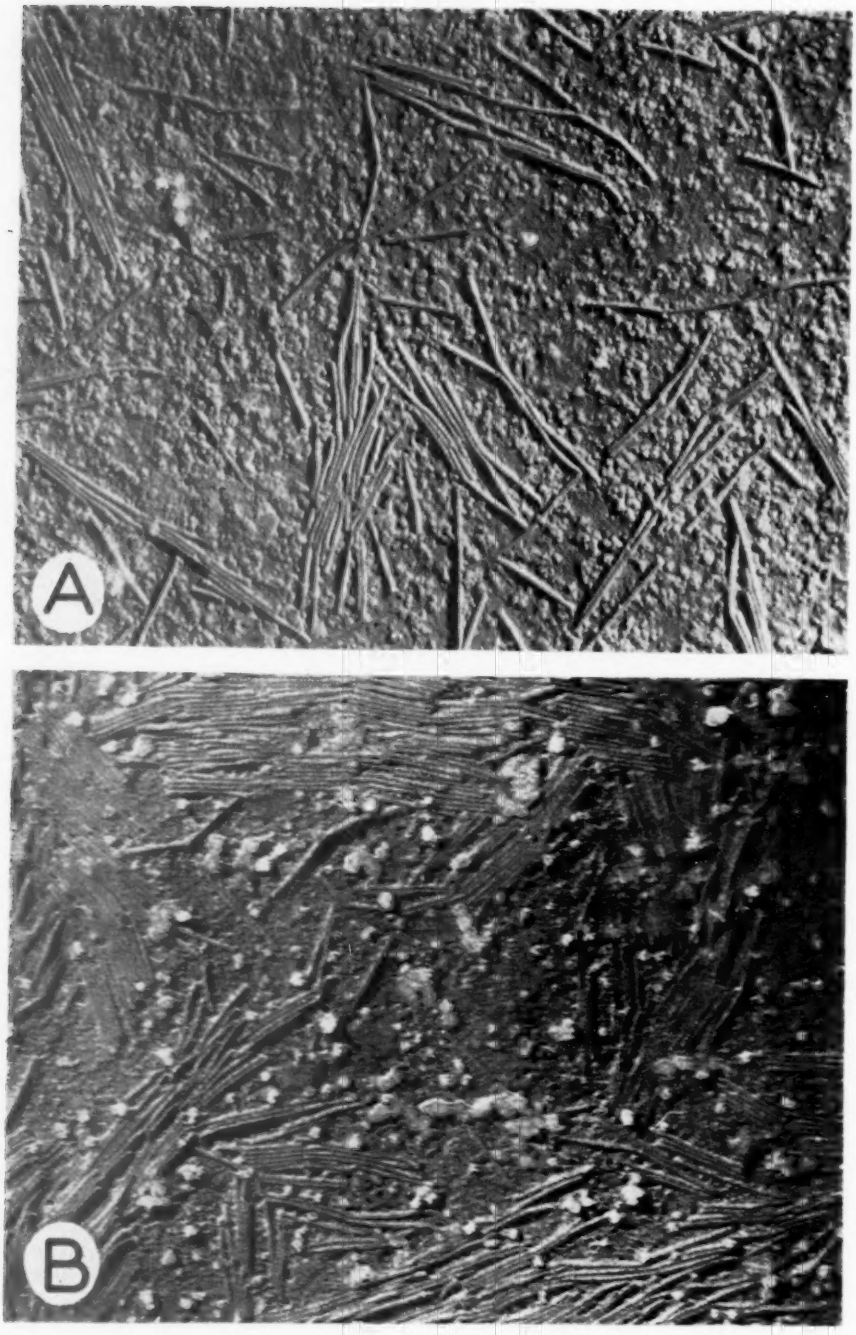

FIG. 3. A. Rodlike particles of tobacco-mosaic virus from tomato, shadow-cast with uranium, showing faint beadlike structure of the rods. (×36,000.) B. Particles of tobacco-mosaic virus from *Physalis lanceolata* with a parallel aggregation of rods. (×37,000.)

N. tabacum L., *N. glutinosa* × *N. tabacum* F_1; *Petunia hybrida* Vilm.; *Physalis aequata* Jacq., *P. angulata* L., *P. floridana* Rydb., *P. lanceolata* Michx., *P. peruviana* L., *P. pubescens* L., *P. virginiana* Mill.; *Schizanthus wisetonensis* Low. (Fig. 4, A); *Solanum nigrum* L., *S. dulcamara*, *Solanum* sp.;[2] *Lycopersicon esculentum* Mill., *L. hirsutum* Dun.; *Nicandra physalodes* L., *Hyoscyamus niger* L. (Fig. 4, B), *Browallia speciosa* Hook., and *Capsicum frutescens* L. They also were found in non-solanaceous species: *Daucus carota* L., *Digitalis lanata* L., *Eryngium aquaticum* L., *Phlox drummondii* Hook., *Plantago major* L., and *Zinnia elegans* Jacq. Thus we have seen the particles in 30 species including 16 genera. Tobacco-mosaic virus particles have previously been reported by others from phlox (*P. drummondii*), tomato (*Lycopersicon esculentum*), and tobacco (*N. tabacum*); and rodlike particles from the latent potato virus (13) and from cucumber viruses 3 and 4 (10).

It became clear early in these studies that all species susceptible to the tobacco-mosaic virus yielded rodlike virus particles, some in much greater numbers than others. The correlation of these observations with those of Hoggan (3), who found intracellular inclusion bodies and striated crystalline plates in the cells of various species infected with tobacco-mosaic virus, may be significant.

We have not yet proved any consistent influence of the host on the size, shape, or structure of the rod-shaped particles. It is clear, however, from the micrographs taken at the same magnifications that considerable variation in width and shape occur, and that some hosts yield virus particles averaging much greater length than others, those of pepper, for example, being more uniformly short. The variations in length in any single host were, however, commonly too great for much importance to be attached to this character, although other investigators (10, 11, 12) give significance to such linear measurements.

It seemed possible that the various strains of the ordinary tobacco-mosaic virus might vary somewhat in particle size or shape. Several single-lesion strains of the tobacco-mosaic virus have been compared, including especially a yellow strain, a mild green strain, and a severe green strain; but no consistent difference in the particles has been noted. The great variation in particle length in all strains has discouraged attempts to demonstrate strain differences by actual measurement.

It was of special interest, however, to observe particles of the yellow strain of tobacco virus in *S. dulcamara*, *Zinnia*, and *Daucus*. These species are apparently very resistant to systemic invasion by other strains of the tobacco-mosaic virus but permit a slow progress of infection with a yellow strain of the virus. In the carrot especially the virus remained at low concentration, thus showing high resistance to multiplication in infected tissues. The virus particles not only were present in infected portions of these three

[2] U. S. Dept. Agr., Introduction P. I. No. 155391 (potato-leaved, tomato-fruit form).

FIG. 4. A. Tobacco-mosaic virus particles from *Schizanthus* illustrating considerable uniformity in size and shape of rods. (×34,000.) Particles normally tend to be scattered irregularly rather than aggregated parallelly. B. Tobacco-mosaic virus particles from *Hyoscyamus*. An unusual type of aggregation, suggesting how crystalline masses may break up into rods of relatively uniform length. (×34,000.)

species, but were similar in size and shape to those in highly susceptible species.

It seems possible that such variations as occur in size and shape of tobacco-mosaic virus particles might be influenced by the plant organ or tissue from which the sample is obtained. The particles obtained from tomato hydathodes, for example, appear relatively more uniform than those obtained from cut ends of veins of tobacco. However, no consistent significant variations were observed in the particles obtained from vascular contents of fibrous roots, stems, petioles, midribs, leaf veins, calyces, petals, stamens, pistils, ovaries, young seed pods, placentae of old seed pods of tobacco, or the fleshy root of carrot.

This extensive search for virus particles, in many cases in organs having no disease symptoms and on hosts very refractory to virus development, was made because of the possible chance of finding infective virus in the absence of virus particles. However, the rodlike particles were invariably associated with the virus of tobacco mosaic.

The numbers of virus particles are, nevertheless, believed to be significantly influenced by the host species or plant organ concerned. The virus concentration and the particle concentration were extremely low in many species or organs so that repeated samples and intensive search of many screens were required to prove the presence of particles. The investigation of virus particles in flower parts by the water-pressure method seems especially to offer possibilities for study of the relationship of numbers to virus infectivity. The frequent lack of good agreement between the amount of virus indicated by local-lesion inoculation and the number of rod-shaped particles observed under the microscope is believed to be of considerable significance. It has been clear throughout this investigation that neither the quantitative relationship nor the correlation between infective units and virus particle numbers was good. For example, the local lesions obtained from one drop of cell contents from the cut veins of a mosaic tobacco leaf were on the average about 100 in number, whereas the number of virus particles in one drop was estimated at 100,000 to 3,000,000. In samples from other species or organs where the number of infective units was low (1 to 10 lesions per drop), the particles were correspondingly few in number and not a thousandfold greater than the number of local lesions. If it is now assumed that the relatively low number of infective units indicated on the test plants is due to the failure of all but an extremely small part of the particles to infect, then the particles should nevertheless be visible on the screen in numbers approximating 1,000 to 30,000 and consequently should not be difficult to detect. The local-lesion hybrid host used is capable of measuring infectivity of ordinary leaf extract up to a dilution of 1–10,000,000. The relatively enormous lack of agreement between particle numbers and infectivity seems theoretically unsound on the basis of cause and effect. Until more is known, however, about the relation of infective units to local

lesions, actual counts of virus particles for statistical analysis with host species or plant organs do not seem to be justified.

Considerable interest centered around the possibility that the virus particles might fail to develop in such rapidly virus-necrotized tissue as occurs in the hybrid host where normal systemic invasion does not occur. To test this assumption, tobacco-mosaic virus at a dilution of about 1–1000 was wiped on 1-in.-wide strips parallel to, but about 1 in. from, the midribs of leaves of the hybrid host. The excess inoculum was immediately rinsed off the leaves with water. As a consequence of the virus activity, the inoculated areas collapsed completely in 40–48 hr. The midrib of a necrotic leaf was then inserted into the water-pressure equipment and the blade of the leaf was cut off with a razor blade through the collapsed band. Droplets of clear exudate were thus forced out of the edges of the necrotized tissue. The exudation was slower than from non-necrotized tissue, but the sample was quite sufficient for electron-microscope purposes when taken soon after collapse and before drying of the leaf. An examination of several prepared screens from such virus-necrotized tissue failed to reveal appreciable numbers of particles of tobacco-mosaic virus. The same method applied to ordinary tobacco, with systemic rather than necrotic symptoms, reveals the particles in abundance.

It seemed likely that the rodlike particles might be filtered out by collapsed portions of the vascular tissue, through which they would need to pass to be present in the exuded droplets. This possibility was tested by collecting droplets from heat-killed tissue of systemically infected leaves. Leaves of ordinary tobacco with mosaic symptoms were suspended edgewise and longitudinally over a water bath so that the leaf blade could be lowered into the water to within about 1 in. of the midrib. The submerged portion of the leaf, heated thus to a killing temperature of 60° C. for 10 min., collapsed about 2 hr. after exposure to the air and resembled the hybrid tissue killed by the virus. In the same manner as before, exudation drops were forced out of the cut edges of this heat-killed tissue of the mosaic-infected leaf. Examination under the electron microscope revealed virus particles in considerable numbers, showing that in this case the particles passed through collapsed heat-killed leaf tissue. Despite these results, it still seemed possible that the virus particles might be present, but in lower concentration, in virus-necrotized tissues. Consequently, sufficient samples were prepared from necrotized hybrid leaves (virus being present only in the necrotized portions) for high-speed centrifugation. Electron-microscope screens prepared with this sediment showed rodlike virus particles in quantity. Consequently, this search for a tissue susceptible to tobacco mosaic but totally free from rod-shaped virus particles was also negative.

More details of the structure of the rod-shaped particles appear to be revealed with the virus material as obtained by the water-pressure method than by other methods of preparation. It is logical to suppose that the exterior of the particles in question is very delicate and likely to be modified

by mechanical disturbance. We have noted that gold shadow-casting, furthermore, is more likely to create artifacts than is uranium shadow-casting, which was used exclusively in our later observations. Under good conditions of photographic exposure and especially in enlarged micrographs many rods appear to be made up of rows of closely fitting spheres or discs (Fig. 6, A, B). This structure is uniformly too clear and too frequent to be dismissed as an artifact. It is not yet possible to conclude that these spheres represent the ultimate virus particle.

ROD-SHAPED PARTICLES ASSOCIATED WITH OTHER VIRUSES

The occurrence of particles similar to those of the tobacco-mosaic virus in other distinct viruses was first demonstrated (13) for the latent potato-mottle or potato X-virus. Using the water-pressure method, we have verified these results both with apparently healthy potato plants and with the potato ring-spot virus strain in *Nicotiana tabacum, N. rustica,* and *Datura stramonium.* Rod-shaped particles have also been described for a few other viruses where the true relationship to the tobacco-mosaic virus is not clear, as in the instance of cucumber viruses 3 and 4 (10).

In the present study, good rod-shaped particles were also found associated with the virus obtained from the vascular contents of the host plant named; mosaic on *Amaryllis hippeastrum* (Fig. 5, A), mosaic on crimson clover, *cucumber virus 4* on cucumber, Dianthus mosaic, mosaic on garden peas, potato latent virus on potato and on *Datura stramonium,* potato ring spot on *Nicotiana rustica,* tobacco ring spot, and tobacco streak on tobacco and on corn. Rodlike particles, either few in number or doubtful in form, were found in a mosaic on alfalfa, ordinary cucumber and yellow cucumber mosaic on tobacco, mosaic on gladiolus, tobacco etch, tobacco yellow ring spot, and turnip mosaic on horseradish.

Repeated trials have failed to determine positively the presence or absence of rods associated with the tobacco ring-spot virus. Fourteen separate trials produced an occasional rod in six samples and none in eight others. Spherical bodies have previously been described for tobacco ring spot; consequently our observation is still further in doubt. Also to be classified among the doubtful cases are alfalfa mosaic, gladiolus mosaic, tobacco etch, and yellow and ordinary cucumber-mosaic viruses. The one of most interest in this group is that of ordinary cucumber mosaic on tobacco, for the reason that the symptoms closely resemble those of tobacco mosaic and may consequently often be confused with the latter virus. This disease is normally free from rod-shaped particles and has been shown not to yield inclusion bodies and crystalline plates (3). Nevertheless, the presence of an occasional well-formed rod in this virus may be significant, as bearing on the origin of such particles without proportional relationship to infectivity. Examination of 19 other virus diseases under the electron microscope by the same method revealed no rod-shaped particles. This group included aster yellows on aster, bean yellow mosaic on garden bean, chrysanthemum stunt,

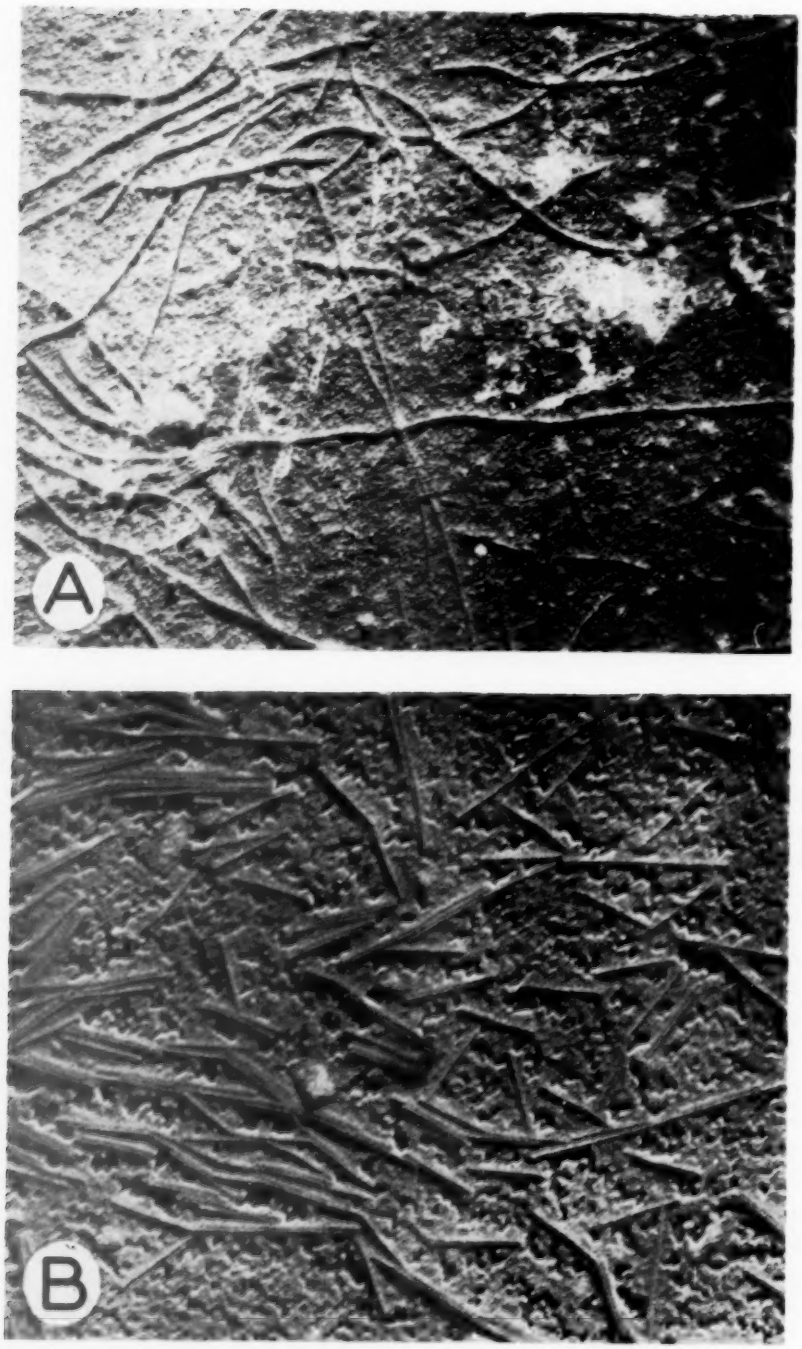

FIG. 5. A. Rodlike or threadlike particles of the Amaryllis mosaic virus. Particles were usually of relatively great length, with some indication of beadlike structure as in tobacco-mosaic virus. Similar long but less numerous particles were associated with several other viruses. (×34,000.) B. Tobacco-mosaic virus particles collected directly from the corolla of the tobacco flower by the water-pressure method. (×35,000.)

a mosaic on common motherwort, cherry necrotic ring spot on cherry and on cucumber, cucumber mosaic on cucumber, dahlia mosaic, dahlia ring spot,

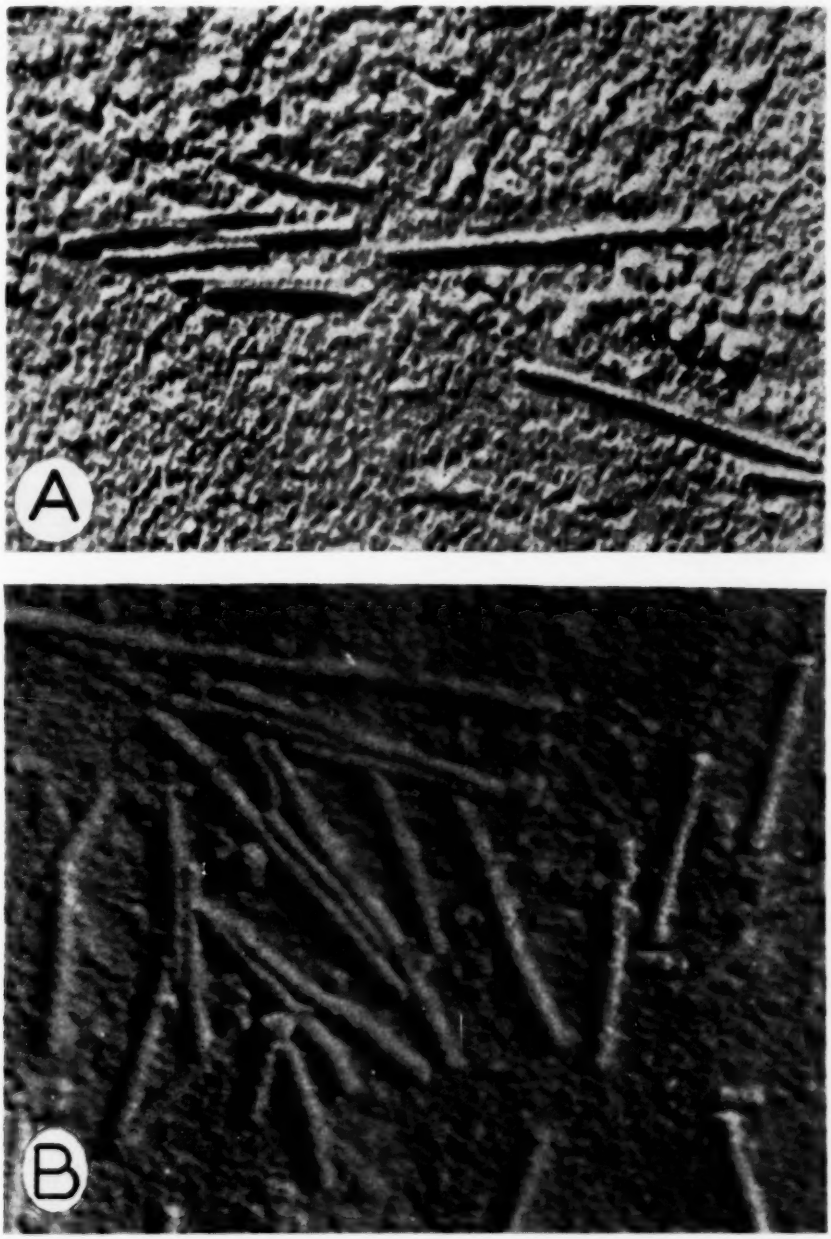

FIG. 6. A. Tobacco-mosaic virus particles obtained directly from placenta of tobacco seed pod showing beadlike detail of rod structure and the type of other materials present in the contents of the vascular system. (×84,000.) B. Tobacco-mosaic virus particles from an unknown species of *Solanum* illustrating the particulate structure of the rodlike bodies themselves but more suggestive of protuberances than beads. (×91,000.)

a mosaic on delphinium, a mosaic on ornamental lupine, potato leaf roll on *Physalis angulatum*, potato yellow dwarf on *Nicotiana rustica*, a mosaic on raspberry, a mosaic on rose, strawberry yellows, a mosaic on squash, tobacco necrosis on tulip, tomato spotted wilt on tomato, and tulip mosaic. These negative results are not to be regarded as conclusive for the entire plant since only the vascular contents were examined and these were not tested for infectivity on their respective hosts.

Thung (15) has illustrated the spherical bodies characteristic of the tomato bushy-stunt virus in vascular contents as well as rodlike particles in potato X, tobacco mosaic in tobacco, the tobacco "rattle disease," and "stengelbout" virus of potato. We have observed spherical particles in the vascular contents of a mosaic disease of squash similar to those described for squash mosaic (14). Minute cubical crystals lying free or in small groups were common in many of the specimens examined. Otherwise the absence, in all the material examined, of other particulate structures that are not also associated with normal vascular contents is striking.

DISCUSSION

Much of the earlier interest in the present investigation centered around the method of obtaining virus particles for electron microscopy by the use of water pressure. The presence of virus determined by inoculation methods in guttation water and exuded water from wounded tissues was reported in 1924 (4) and in 1937 (6) from this laboratory. It was natural that such material should be examined under the electron microscope, but so far as is known it has been done independently elsewhere only by Thung in Java in 1948, with results similar to ours (8). The ease and rapidity of the water-pressure method for collecting vascular contents permits the examination of a large number of different samples under the electron microscope without the comparatively long and laborious extraction and purification required by other methods. Where a number of different species or samples are to be compared, the water-pressure method is apparently more reliable, simple, and uniform than other short methods that have been described (11). We have been able to prepare and examine several hundred samples of material, obtained from a variety of plant species (including many glaucous forms) as well as from various plant organs or tissues. Quite as interesting as the search for virus particles has been the observation of the normal material present in the vascular contents, including amorphous masses and cubical crystals. These would seem to offer opportunities for extensive study on the physiology and nutrition of plants.

After the technique had been developed and the material most suitable for study had been found, the opportunity to observe material from a variety of sources caused a shift of interest in the investigation to the size, shape, and structure of the rodlike virus particles themselves. It was, first of all, obvious that the variation in length of the rodlike particles was so great that measurements would be of little significance. Particles no longer than their

width to those longer than two diameters of the microscope field have been observed, although fairly uniform "typical" lengths were common in many instances. It is conceivable that the rods are beadlike strings (Fig. 6, A) that may break at the weakest point. The particles clearly have much uniformity in width in the same field, though rods only half as wide as others were often seen where different samples were compared at the same magnification. The rods were often straight and appeared as rigid as a crystal; less frequently they appeared flexible, bent, or threadlike, and rarely were they branched. We saw no evidence that any of the material resembled living organisms. That these relatively large and variable rod-shaped particles are to be regarded as chemical molecules representing the ultimate virus unit also seemed unlikely. This viewpoint appears to be supported in part by the absence of uniform particulate forms of any type in the electron microscopic field in the instance of several distinct viruses. Particle aggregates of the tobacco-mosaic virus resemble minute crystals and are apparently of the same type as the striate material described and illustrated by Hoggan for tobacco mosaic (5). This suggestion has been previously made by Beale (2). The belief is further supported by the frequent occurrence of striated crystalline "plates" in the exudate obtained directly from vascular contents (Fig. 2, C). Though its origin is more in doubt, the flexible and threadlike material may represent some intermediate stage in the development of the more rigid particles.

A significant part of this investigation is concerned with the relationship between the number of rodlike particles present on the microscope screen and the number of local lesions that may be obtained with a comparable amount of virus on inoculation to the highly sensitive hybrid host, *Nicotiana tabacum* × *N. glutinosa*. We have estimated that over 100,000 and possibly 3,000,000 particles are present on a single screen representing only about one-fourth of a drop of tobacco-mosaic-virus exudate from the vascular system. Nevertheless, comparable inoculation with whole drops to the hybrid host may average less than 100 local lesions to the drop. As is well known, the percentage of the virus particles causing infection is extremely low. Although this may be regarded as evidence of relatively low susceptibility on the part of the host, it has frequently been shown that the hybrid will yield a few local lesions at a 1–10,000,000 dilution of mosaic plant juice extracted in the ordinary manner. The discrepancy and lack of agreement between particle number and infectivity becomes more obvious as different samples of virus from different sources are compared. It may also be deduced from many comparative tests that the rod-shaped particles obtained from the vascular contents are relatively noninfectious, even immediately after being removed from the diseased host plant. On the other hand, since we have found virus particles in equally high numbers in old cured mosaic-infected tobacco leaves that had lost most of their infectivity, the particles are evidently more resistant to destruction than is any other form of the virus.

It is believed that the water-pressure method of securing virus for elec-

tron microscopy offers a more uniform and less drastic means of collecting the virus than other methods that have been used. Consequently, the relation of particle aggregation and disruption to the size and shape of virus particles may be more reliably investigated. Our studies have not been chiefly concerned with this problem, as have those of others (10, 11, 12). However, the observation of a large variety of species and organs involving close to 200 separate preparations does not show the uniformity of size and shape of the rodlike particles comparable to that of either single-cell units of microorganisms or conceptions of single molecules. The investigations, on the other hand, point rather toward the conclusion that the rod-shaped particles are a result rather than a cause of disease. That the particles in question contain or are made up of smaller virus units, as is believed by Bawden (1), appears to be a necessary conclusion to explain the activity attributed to their presence. More consideration to Koch's postulates, or the equivalent, may be required to finally determine the true nature of the ultimate virus units.

SUMMARY

Through the use of water pressure on virus-infected plant tissues, clear exudates were obtained from about 50 plant species infected with about 35 distinct viruses. These drops were transferred directly to microscope screens and prepared further for electron-microscope examination.

The rodlike particles typical of tobacco-mosaic virus were found in 30 species of host plants, six of which were nonsolanaceous plants. The particles were also found in practically all tissues of certain species, including the roots, petals, stamens, and pistils.

Similar rodlike or threadlike particles were found associated with several other distinct viruses, but rods were clearly absent in another large group of virus-infected plants. The shape and size of the particles were more variable among the different viruses than they were with the tobacco-mosaic virus strains on different host species or plant organs.

The number of virus particles observed under the microscope was often roughly in proportion to the number of infections obtained on a local-lesion host, but this agreement was much less pronounced in some species or organs than in others. Quantitatively, the particles are normally tremendously in excess of the infectious units as measured by the local-lesion method on a highly sensitive host.

Considering the variable size and shape of the particles, their numbers relative to infectious units, and their similarity when in parallel aggregation to striated crystalline plates visible under the light microscope, it does not seem likely that they represent the ultimate infectious unit of the virus. At high electron-microscope magnification and by means of uranium shadow-casting, the rods of the tobacco-mosaic virus resemble a row of thick discs or spheres. These observations together with others, such as the absence or rarity of visible particles in other similar plant viruses, suggest that the

rodlike particles in question may be a result of disease rather than the ultimate causal unit.

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THE VIRUS OF TOBACCO LEAF CURL. II

D. G. SHARP AND F. A. WOLF[1]

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Rod-shaped particles were found by the authors in formolized sap of tobacco plants affected with leaf curl (3). Although these particles strongly resembled those of ordinary tobacco mosaic virus, it was thought they were the infectious agent of leaf curl. Subsequent work has shown that this assumption is probably incorrect. Serological tests[2] made with some of the same formolized material used in the previous work indicated the presence of the mosaic virus in these preparations, and when tobacco seedlings were inoculated[3] with it symptoms of mosaic developed. Although an admixture of leaf-curl virus in these materials might be assumed, no viruslike bodies, other than those reported, were detected by the physical means employed.

Infectivity tests with leaf-curl virus are limited by the fact that its transmission depends upon the activities of white flies. This fact was shown by our own pathogenicity tests in Venezuela (6), which confirm studies of transmission of leaf-curl virus made in other parts of the world. At present, with a given mixture of viruses in sap, no satisfactory procedures are known for establishing the causal relation to disease of viruses like the tobacco leaf-curl virus. It seemed best, therefore, to secure additional samples for study, using sap from plants proved to be free of ordinary tobacco mosaic virus, rather than to use a mixture of viruses. To this end, samples from Venezuela were supplied to us by Dr. C. H. Meredith, Dr. J. H. Standen, and Mr. Emery Hooper, to each of whom we herewith extend thanks. Portions of these samples were tested, first of all, to determine by inoculation whether or not they contained mosaic virus. All were free of it, and certain of them were found to contain particulate material of a kind not known to occur in healthy tobacco plants. The results of these further examinations of formolized sap samples are presented to clarify and augment the findings from the previous report (3).

GENERAL EXPERIMENTAL PROCEDURE

The formolized plant sap was clarified by centrifuging for 15 min. at 3,000 G in an angle centrifuge operating in the cold room (0° to 4° C.). The supernatant fluid was removed and ultracentrifuged for 60 min. at 47,000 G. The resulting pellet was next resuspended in the original volume, using 0.06 M phosphate buffer pH 7.2, and this suspension was spun again

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[2] The serological results were obtained through the courtesy of Dr. K. M. Smith of the Molteno Institute, Cambridge University.

[3] These tests were expected to be futile because of the age of the sample and the presence of formaldehyde, but they demonstrated active mosaic virus in spite of the unfavorable conditions.

at low speed (3,000 G) for 15 min. to remove such material as had not redispersed. This supernatant fluid was then ultracentrifuged a second time at 47,000 G for 60 min. and this final pellet was taken up in phosphate buffer. In this process the overall concentration, ignoring losses, was 20 times by volume, and it was at this concentration that sedimentation velocity studies were carried out. For electron microscopy, dilution was necessary, as will be indicated. The above procedure differs from that used previously (3) in two seemingly unimportant details. The phosphate buffer was 0.06 molar rather than 0.1, as before, and the ultracentrifuge was run at 47,000 G instead of at 25,000 G.

SEDIMENTATION VELOCITY STUDIES

The same vacuum-type, air-driven ultracentrifuge was used for these studies as had been employed in the earlier ones, but, since the amount of material was limited, sedimentation velocity measurements were made at a single concentration only. Two sedimenting boundaries were observed. The greater part of the material sedimented as a homogenous component, yielding a sedimentation constant of S = 75 in the first run and S = 74 in the second. Part of the material formed a second, more diffuse boundary with sedimentation constants in two runs of S = 112.7 and S = 112.9, respectively. This faster-sedimenting fraction was estimated to constitute about 10 per cent of the total. Neither boundary was as sharp as is commonly seen in preparations of tomato bushy stunt virus or even those of tobacco mosaic virus.

ELECTRON MICROSCOPY

The concentrate to be examined with the electron microscope was diluted 1–10 with water, deposited on collodion and chromium-shadow-cast at an angle of 15°. The appearance of the particles in this concentrate is shown in figure 1. The approximately round single images in this picture measure about 39 mμ. The shadows are very short, indicating flattening of the particles on the collodion film. Heat and desiccation during the shadow-casting operation may have caused this flattening. They appear to be crenated and shrunken to an extent even greater than that commonly seen with the larger animal virus particles similarly prepared.

Less clearly shown but more numerous particles are seen in a more concentrated (1–4 dilution of concentrate) preparation (Fig. 1). Here again the image size is that of bodies about 39 mμ diameter, and the flattened crenated appearance can be observed. All preparations studied appear to be composed almost entirely of one kind of particle, occurring either singly or in aggregates.

DISCUSSION

These preparations from sap of leaf-curl-affected plants free from tobacco mosaic virus have predominantly one kind of particle, which is unlike

ordinary mosaic virus in appearance and in sedimentation behavior. The particles differ also from other essentially spherical tobacco viruses that have been described previously. So far as is known, only two tobacco viruses have comparable sedimentation constants, and these are the viruses

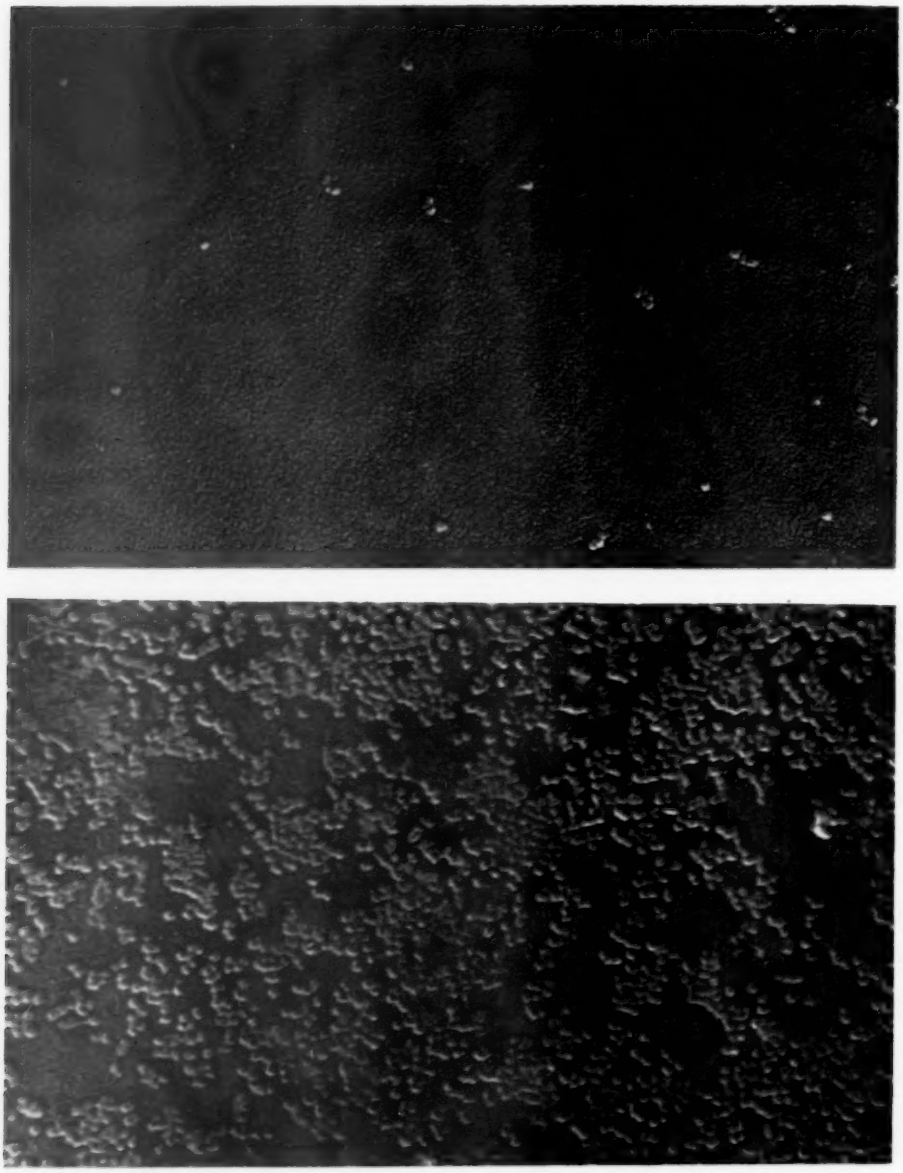

FIG. 1. Purified concentrate of particulate material isolated by ultracentrifugation from formolized sap of tobacco plants affected with leaf curl. Upper: In 1–10 water dilution, dried on collodion and shadow-cast with chromium at an angle of 15°. Single round bodies are 39 mμ in diameter. (39,000×.) Lower: In 1–4 water dilution. The particles cast exceedingly short shadows, indicating considerable flattening on the supporting membrane. (40,000×.)

of tobacco necrosis and tobacco ring spot, for which the values are S = 112 (2) and S = 115 (5), respectively. Leaf-curl material has a faint component in this region also, but this might arise from pairs of particles whose sedimentation constant as individuals is S = 75, the observed value for the principal component in these preparations. It seems probable that those two sedimenting boundaries are two aspects of the same material rather than that there is present a mixture, one component of which is either ring-spot virus or necrosis virus.

From sedimentation rate data the diameter of the sedimenting unit can be calculated, once the shape is known, but such procedure must be based upon a knowledge of the density of this unit. Partial specific volume data are useful only in calculating particle weight exclusive of associated water or solvent, but wet density is necessary for size calculations. Wet density is not available for any of the three materials under discussion, yet, if one assumes such a value for the leaf-curl material to be 1.33[4] and the shape to be spherical, the corresponding particle diameter is 20 mμ. On the other hand, if the sedimenting hydrated unit particle is more like that of certain recently studied animal viruses (4) its density may be as low as 1.1, in which case the diameter would be 37 mμ. This point will be discussed later in connection with the electron microscope findings.

The appearance, in the electron micrographs, of the chromium-shadow-cast particles of the leaf-curl material is that of approximately round, flattened, and somewhat indented bodies. There seems to be present little extraneous material, and no rodlike bodies were seen. Single round images indicate a diameter of 39 mμ. Various small-number aggregates seen in figure 1 may account for the diffuse secondary boundary (S = 113), and possibly for other faster-sedimenting ones not observed in our experiments but which may nevertheless exist. These images are undoubtedly those of the particles causing the sedimenting boundaries, but their shadows are exceedingly short indicating excessive flattening during preparation for examination with the microscope. Structural weakness which permits excessive flattening, common among the larger viruses from animals, has not been reported previously with plant material. Flattening may, in fact, be evidence of high water content, such as that possessed by particles of soft structure in water suspension with wet density near the 1.1 value. It is difficult to predict whether such a structure, after having been desiccated and flattened, would yield an image diameter greater or less than that of a particle suspended in cell sap or in aqueous media. In any case, this true value of particle diameter is probably not much different from 39 mμ, a size substantially greater than that attributed to tobacco necrosis virus (1, 2) or to tobacco ring-spot virus (5).

The amount of particulate material obtained is roughly comparable to the amount of ordinary tobacco mosaic virus which one obtains from plants

[4] This is the reciprocal of the value 0.75 frequently found for the partial specific volume of plant viruses.

affected with tobacco mosaic. But, had tobacco mosaic virus been present in the formolized sap employed herein, it should have appeared. The material is sufficiently stable to retain the physical properties shown in the micrographs, in spite of formolization and shipment in crude form from Venezuela.

It may be presumed that the spherical particles obtained from formolized sap of leaf-curl-affected tobacco plants constitute the leaf-curl virus. To obtain positive proof that such is the case, however, seems impossible under present conditions. Even if one were in an area where tobacco leaf curl is endemic and had at his immediate disposal the necessary mechanical equipment, there would still remain the problem of contaminating or infecting the white-fly vector with the given virus that had been mechanically isolated and purified. Until techniques to do so have been developed, essentially all that can be done to prove the relation of virus to this leaf curl is to isolate repeatedly similar particulate material from leaf-curl-affected plants. In due time, no doubt, investigators will have done so, and a body of evidence will have been accumulated as to the physical appearance of the agency responsible for tobacco leaf curl.

SUMMARY

A particulate material, physically unlike other tobacco viruses, has been isolated by ultracentrifugation from the formolized sap of leaf-curl-affected tobacco plants obtained from Venezuela. Its sedimentation constant is S = 75 units. Electron micrographs of this material show great numbers of substantially one kind of image. The particles seem to be soft, approximately spherical bodies which become flattened upon the supporting film during preparation for examination with an electron microscope. The mean image diameter for unaggregated particles is 39 mμ.

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PATHOLOGICAL NOTES

Phytophthora Root Rot of Tomatoes.[1] J. P. Fulton and Neil D. Fulton. Rapid wilting and death of 36 plants in a circular area in a field of tomatoes of the variety Fortune[2] planted on the Experiment Station Farm at Fayetteville, Arkansas, were noted in mid-July of 1949. A few diseased plants were found scattered along the drainage path from the area. *Phytophthora parasitica* Dast. was consistently obtained when stem pieces from diseased plants were plated on potato-dextrose agar. Richardson[3] reported a similar disease of tomatoes in Canada caused by this pathogen. Prior to that Reddick[4] reported its occurrence in New York. In both of these cases the disease occurred primarily on greenhouse tomatoes. Although Richardson reported its appearance in one outdoor crop, he was apparently concerned with the damping-off of young plants. A disease with similar symptoms was reported from England by Pethybridge and Lafferty,[5] although the causal agent is undoubtedly a different fungus (*Phytophthora cryptogea* Pethyb. & Laff.).

The first wilted and dying plants were noted during the first dry weather of the growing season. The diseased plants resembled those affected by bacterial wilt in that no yellowing accompanied wilting and death. The pith of the stem near the ground line usually had a brown decay, and browning of the vascular system sometimes extended throughout the plant. No exudate, as in the case of bacterial wilt, was present. Roots were so extensively rotted that the cortex sloughed away and in most cases all that remained was the whitened, dry vascular system.

The fungus grew readily on a variety of culture media and its cultural characteristics were essentially the same as those listed by Richardson and by Tucker[6] for *P. parasitica*. Growth was luxuriant on oatmeal agar and chlamydospores developed in old cultures. Sporangia appeared only occasionally. However, when pieces of oatmeal agar on which the fungus was growing were transferred to sterile water the sporangia were abundant and each sporangium produced 50 to 85 zoospores. When these sporangia were transferred to fresh water, the discharge of zoospores began in a few minutes. Oospores were observed in one inoculated flask of sterilized oat grains after about 2 months.

[1] Research paper No. 930, Journal Series, University of Arkansas. Published with the permission of the Director of the Arkansas Agricultural Experiment Station.

[2] A Fusarium wilt-resistant variety developed by Dr. V. M. Watts and released by the Arkansas Agricultural Experiment Station in 1949.

[3] Richardson, L. T. A Phytophthora disease new to Ontario. Canad. Jour. Res. (C) **19**: 446–483. 1941.

[4] Reddick, D. A fourth Phytophthora disease of tomato. Phytopath. **10**: 528–534. 1920.

[5] Pethybridge, G. H., and H. A. Lafferty. A disease of tomato and other plants caused by a new species of Phytophthora. Sci. Proc. Royal Dublin Soc., n. s., **15**: 487–503. 1919.

[6] Tucker, C. M. Taxonomy of the genus Phytophthora de Bary. Mo. Agr. Exp. Sta. Res. Bul. 153. 1931.

The cultural characteristics of this fungus were compared with two other isolates of *P. parasitica* obtained from green tomato fruit having typical buckeye-rot symptoms. One of the two had been identified by Dr. C. M. Tucker as *P. parasitica*. These three isolates appeared to be identical in microscopic and cultural characteristics.

Inoculum containing motile zoospores proved to be consistently virulent when greenhouse tomatoes were inoculated. Oatmeal agar cultures were cut into small squares and placed in a large dish containing a thin layer of sterile water. Sporangia appeared within 48 hr. and the preparation remained satisfactory for use as inoculum for several weeks. The material from one of these dishes was briefly macerated with additional water in a Waring Blendor. The maceration largely removed any evidence of the mycelium, but the sporangia remained uninjured. Tomato plants to be inoculated were removed from the soil and dipped in the inoculum, or a small amount of inoculum was poured around the base of the roots. The plants were grown in a potting mixture of 2 parts field soil, 1 part rotted manure, 1 part sand, and 1 part puffed mica. This mixture was sterilized in a soil sterilizer constructed with electric heating elements. The greenhouse was operated at approximately 80° F.

Since the disease appeared on Fortune, a new variety of tomatoes, it was of primary interest to determine whether it also attacked such varieties as Rutgers, Bonny Best, Marglobe, and Stokesdale. Plants of each variety were inoculated at various ages ranging from small plants with only one true leaf to older plants with 6 to 7 true leaves. No consistent difference could be demonstrated in the susceptibilities of any of the five varieties used. Richardson studied 40 commercial varieties and found all to be completely susceptible.

Symptoms developed most rapidly on young plants as a very rapid rotting and collapse of the taproots and stems. Older plants were most often attacked either at the tip of the taproot or at the ground line. Rotting of the taproot tip appeared more consistently on inoculated specimens than the collar-rot or damping-off symptom. In some cases the rot progressed through the pith up into the stem, which collapsed, and led to the death of the plant. In other cases the rot was limited to the lower inch or two of the taproot; above this portion there was excessive development of lateral roots. From the rotted portion of the root a vascular discoloration extending several inches up the stem was noted on some specimens, but the extensive discoloration observed in some diseased plants from the field was never duplicated in the greenhouse. Discoloration of the vascular system was apparently caused by deposition of brown material in the vessels and was not related to invasion of the vascular system by the fungus. None of the greenhouse studies indicated that this fungus was capable of acting as a vascular parasite.

The three isolates of *P. parasitica* were equally virulent on tomato plants of the varieties Fortune and Rutgers inoculated at various ages. No dif-

ference in the production of typical root-rotting symptoms was noted. Young green tomato fruits inoculated with zoospores from the three isolates developed typical buckeye-rot symptoms. There is little doubt that the fungus which rotted the tomato roots in the field is identical with that which causes buckeye rot of the fruit.

Richardson pointed out the reduction in amount of disease in unsterilized soil as compared with sterilized soil. In the present studies consistent results with inoculated plants were obtained only when sterilized soil was used. Plants could be inoculated in unsterilized soil, but symptoms varied from severe to very mild root rotting. The addition of organic matter to unsterilized soil reduced disease development; when added to sterilized soil it increased disease development.

The amount of damage this disease causes in tomato fields in Arkansas is unknown. Since buckeye rot appears commonly on tomato fruit, the pathogen is widely distributed. The greenhouse trials indicated that severe damage to the roots was required before wilting became evident. Mildly affected plants were stunted but otherwise appeared vigorous. It appears likely that this type of plant would be overlooked in the field unless a careful examination of the roots was made.—Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

Strumella Canker on Bur Oak in Pennsylvania.[1] CHARLES L. FERGUS.—In the winter of 1948 young bur oaks (*Quercus macrocarpa* Mich.) were found to be seriously infected with *Strumella coryneoidea* Sacc. & Wint. The bur oaks are part of a 20-year-old mixed plantation, the other species being Scotch pine and Northern White cedar, in the experimental woodlot of The Pennsylvania State College, Centre County, Pennsylvania.

Heald and Studhalter[2] and Bidwell and Bramble[3] have listed the suscepts of *S. coryneoidea*, but this is the first report of the pathogen on bur oak, fulfilling Bidwell and Bramble's prediction that the suscept list for this pathogen would be extended.

Because of the extreme virulence of *S. coryneoidea* on these young bur oaks and because the pathogen had not heretofore been described on bur oak, a more complete survey of the damage incurred was made.

Observations on 260 trees in the stand disclosed that 30 of them had been killed and an additional 100 had Strumella cankers. The trees had not grown well because the soil is a semi-hardpan with poor water drainage, and poor air drainage subjects the area to late spring frost injury. In addition, some trees suffered dieback from an unknown cause or causes; no

[1] Contribution No. 159 from the Department of Botany, Pennsylvania Agricultural Experiment Station. Authorized for publication March 6, 1950, as paper No. 1587 in the Journal Series.

[2] Heald, F. D., and R. A. Studhalter. The Strumella disease of oak and chestnut trees. Pa. Dept. Forestry Bul. 10. 1914.

[3] Bidwell, C. B., and W. C. Bramble. The Strumella disease in southern Connecticut. Jour. Forestry **32**: 15–23. 1934.

correlation could be found between trees with Strumella canker and those with the dieback.

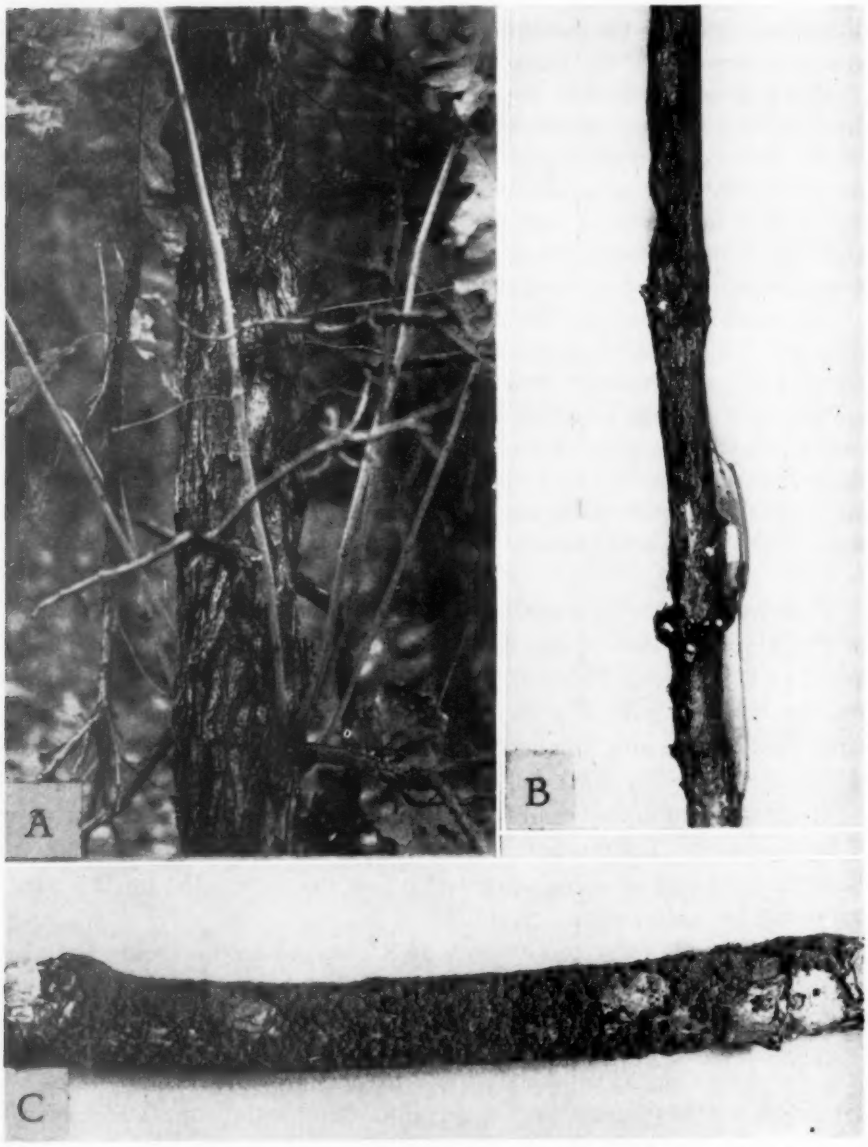

FIG. 1. *Strumella coryneoidea* on bur oak. A. Young canker showing depressed area and water sprouts. B. Bur oak killed by the fungus. C. Sporulation of *S. coryneoidea* on a dead branch.

Young cankers on bur oak are very difficult to detect because of the extreme roughness of the bark. In figure 1, A, a young canker near the center of the illustration has abundant water-sprout formation below the canker. The water sprouts and the slightly depressed area of the bark indicate the

presence of the pathogen. The sprouts are visible for some distance, and were used in making observations on the number of infected trees. It is highly probable, however, that many young sproutless infections were overlooked and that the number of infected trees was greater than the number detected.

The main section of the trunk of a bur oak killed by the fungus is illustrated in figure 1, B. As shown by annual-ring count, the tree was approximately 10 years old when it died and had attained 1½ in. d.b.h.

Dead branches, dead water sprouts, and trunks of killed trees were abundantly covered with masses of black sporodochia. Fruiting of *S. coryneoidea* on a killed side branch (Fig. 1, C) explains the term "gunpowder" for the sporulation, used by lumberjacks. Microscopical examination of the sporodochia and conidia showed the fungus to be *S. coryneoidea*. Isolations from the wood beneath the cankers of killed trees and from trees with young, sunken cankers yielded pure cultures of a fungus which agree in all details with the published descriptions and photographs of *S. coryneoidea*. No inoculation studies have been attempted, but physiological studies of the fungus are being carried out in this laboratory.

The original source of infection for these young bur oaks is not definitely known, but mixed natural stands containing oaks infected with *Strumella* are within 900 ft. of the mixed plantation, and it is assumed that infection came from these trees. Bramble[4] has pointed out the possibility of Strumella infection originating in such a manner.

The bur oaks were planted in 6 × 6-ft. spacings and it is to be expected that with the tremendous increase in number of spores in the young plantation, infection will occur on many more of the oaks.

These observations lend further strength to Bramble's recommendation for control. To avoid infection in the future it would be advisable to remove trees infected with *Strumella* from adjacent stands prior to or coincidentally with the establishment of plantations of oak species known to be susceptible to this disease. Oak species that are supposedly nonsusceptible, when grown in plantations, may prove to be susceptible as has been found with bur oak.—Department of Botany, The Pennylvania State College, State College, Pennsylvania.

[4] Bramble, W. C. Strumella canker in a Northern Red oak plantation. Jour. Forestry **44**: 527. 1946.